# PROLACTIN AND OVARIAN FUNCTION IN THE HUMAN FEMALE

RUNE ROLLAND

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PROMOTOR : PROF. DR. J.L. MASTBOOM

CO-PROMOTOR : PROF. DR. E.V. van HALL

CO-REFERENT DR. L.A. SCHELLEKENS

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AKADEMISCH PROEFSCHRIFT TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN, OP GEZAG VAN DE RECTOR MAGNIFICUS PROF. DR. F.J.F.M. DUYNSTEE VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN IN HET OPENBAAR TE VERDEDIGEN OP VRIJDAG 13 DECEMBER 1974 DES MIDDAGS TE 2 UUR

DOOR
**RUNE ROLLAND**
GEBOREN TE BERGEN, NOORWEGEN

DRUK VAN HOOREN B V HEERLEN

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Contents

## INTRODUCTION

In the past few years, an increasing interest has been shown to prolactin and its possible action in the regulation of endocrine functions in mankind resulting in many good reviews dealing with these problems (Cowie and Tindal, 1971; Ho Yuen et al., 1973; Horrobin(a), 1973; Pasteels and Robyn, 1973; and Sulman (a), 1970). This development is mainly due to the fact that prolactin has been recognized as one separate entity in the human and that methods have been developed sensitive enough to measure its concentration in plasma. Before dealing with the problem of prolactin and ovarian function in the human female, however, it seems reasonable to give a short summary of the history of this hormone and its functions in different species for a better understanding of the different actions in the human.

## PROLACTIN IN VERTEBRATES

The first real evidence that the anterior pituitary produced a mammotrophic hormone inducing copious milk secretion in the mammals was established by Stricker and Grueter in 1928 (see Stricker, 1951). Shortly thereafter, Riddle and co-workers prepared partially purified pituitary extracts demonstrating the same lactogenic response with the concomitant property of stimulating the secretory activity of the pigeon crop sac. His group introduced the name Prolactin (Riddle et al., 1933).

Until 1963, prolactin was solely regarded as a mammotrophic and gonadotrophic (luteotrophic) hormone. However, since the review of Riddle in 1963 in which he emphasized that it should be regarded as having metabolic properties next to reproductive properties, it has become clear that this hormone plays a significant role in various physiological processes (Riddle, 1963). In a review by Nicoll and Bern (1972), 84 different actions of prolactin were reported among cyclostomes, teleosts, amphibians, reptiles, birds and mammals. Unlike other adenohypophyseal hormones, prolactin did not show a specific action for the regulation of one single physiological process. Following Nicoll and Bern (1972), its actions can be classified as follows related to:

1. Reproduction
2. Osmoregulation
3. Growth promotion
4. Ectodermal structures
5. Synergism with steroid hormones or action on organs which are also influenced by steroids.

By using these headings, the described actions of prolactin in mammals regardless of the animal species are shown in Table 1 (L'Hermite, 1973). From this table it is clear that the influence of prolactin on many of these functions can only be secondary and that it has to interfere with other regulatory processes. No common denominator has been demonstrated for the actions of prolactin in the mammals (Nicoll and Bern, 1972). However, the possibility of prolactin to act without interfering with the second messenger (cyclic-AMP) makes it different from other pituitary hormones and could explain its permissive role on the responsiveness of different organs to other endocrine regulatory mechanisms (Horrobin (b), 1973).

**Table 1:** Biological Actions of Prolactin, as Described in Mammals Regardless of Species

**Reproduction**
Mammary development
Lactation
Luteotrophic action
Fertility control
Luteolytic action
Advanced puberty
Decreased copulatory activity
Parental behaviour
Vaginal mucification
Effect on male accessory sex glands
Preputial gland size and activity
Increased androgen binding in prostate
Increased cholesterol in testis
Increased glucuronidase activity in testis

**Osmoregulation**
Lactation
Increased retention of sodium, potassium and water

**Ectodermal structures**
Mammary development
Lactation
Effects on sebaceous and preputial glands
Hair maturation

**Growth promotion**
Mammary development
Male accessory gland development
Luteotrophic action
Spermatogenesis
Erythropoietic effect
Hair growth
Sebaceous and preputial gland growth

**Synergism with steroids**
Mammary growth
Milk secretion
Advanced puberty
Luteotrophic action
Spermatogenesis
Renal retention of sodium, potassium and water
Vaginal mucification
Effects on male accessory sex glands
Sebaceous and preputial glands
Secretion
Hair growth

**Actions like growth hormone**
Lipid deposition and/or mobilization
Hyperglycemic-diabetogenic action
Effects similar to HGH on sodium balance, NEFA and calcium metabolism

From: L'Hermite, **M.**: (1973) The present Status of Prolactin Assays in Clinical practice. Clinics in Endocrinology and Metabolism, W.B. Saunders Company, London, pp 426, with permission. (Minor modifications made by editor)

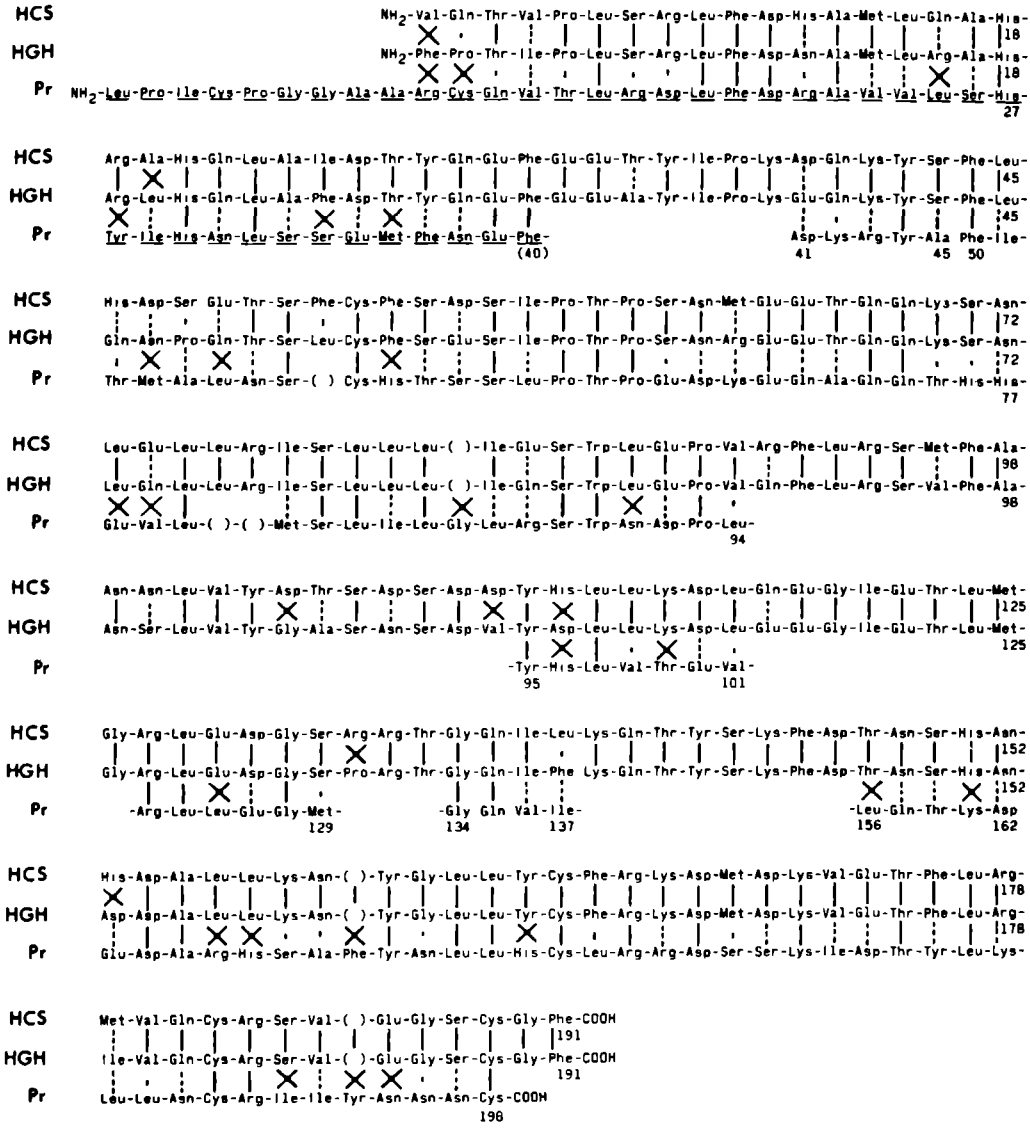
After the demonstration of prolactin in the anterior pituitary of different vertebrates, highly purified extracts of this hormone were isolated from different species (Riddle, 1963). Ovine prolactin was demonstrated as early as 1937 by White and co-workers, and its molecular structure has recently been described (Li, 1972). However, although there was a strong belief that prolactin also existed in the human, direct evidence could not be found. Indirectly, prolactin was demonstrated by the rather unspecific pigeon-crop-sac assay and later on by more specific in vitro bioassays using induction of different enzymes or amino acids in mammary tissue from different animals. The quantitative in vitro bioassay of Loewenstein with co-workers (1971), inducing N-acetyllactosamine synthetase by prolactin, was especially useful. This enzyme is necessary for the transformation of N-acetyl-glucosamine and UDP-galactose to N-acetyllactosamine. The quantitative in vitro bioassay using the amount of produced $^{32}$P-caseine, a prolactin dependent function, was also very useful (Turkington, 1971).

Both Human Growth Hormone (HGH) and Human Placental Lactogen (HPL) exhibit lactogenic activity in all developed bioassays, and it was doubted by different investigators if prolactin really existed as a separate entity at all. All differences in growth promoting or lactogenic activities could be due to chemical degradation during purification of one single homogeneous protein with both activities intrinsic to it (Chadwick et al., 1961; Apostolakis, 1968; Forsyth, 1969; and Lyons, 1969).

At the same time during 1960 to 1970 an increasing amount of evidence was collected favouring the view of one prolactin next to HGH and HPL. The experiments of Pasteels and co-workers were convincing in which they showed that prolactin producing cells in the human pituitary existed and also that when pituitary cells were cultured in vitro, HCG-activity decreased with an increase in the lactogenic activity (Pasteels, 1963, 1969). Histologically, a difference between somatotrophic cells and prolactin cells was found using the tetrachrome staining technique of Herlant (1960) in human pituitaries. The prolactin cells were very scarce except during pregnancy and lactation (Herlant and Pasteels, 1967). Lactation was also reported in dwarfs with isolated HGH deficiency (Rimoin et al., 1968), and low serum levels of HGH were found in conditions associated with galactorrhea (Benjamin et al., 1969). Also, pituitary tumors were reported to be rich in a material causing prolactin-like activity in different bioassays without an increase in the amount of measurable HGH (Takatani et al., 1967; Peake et al., 1969). The inactivation of the lactogenic activity in human blood samples with an antiserum against ovine prolactin, but not with an antiserum against HGH, finally pointed out prolactin as a separate protein entity (Frantz et al., 1972 (a)).

Incubation studies with rat anterior pituitary glands and radioactive amino acids demonstrated a release of prolactin from the glands into the culture medium (MacLeod et al., 1969; Birge et al., 1970). When doing these studies with monkey and human pituitary glands, Friesen's group was the first one to demonstrate a similar protein in these two species next to GH. Also, the chemical amount was extremely small compared to HGH (Friesen et al., 1970). However, the biosynthesis of human prolactin had been demonstrated and very soon thereafter the hormone could be further isolated in larger amounts (Lewis et al., 1971 (a); Hwang et al., 1972). As expected, because of observed immunological cross reaction, human prolactin is very closely related to ovine prolactin consisting of one peptide chain of 198 amino acids with three disulfide bridges and a molecular weight of about 23,000. HGH and HPL (HCS) have both a single peptide chain of 191 amino acids with two disulfide bridges which are found at the same amino sequences

Fig. 1




Comparison of the amino acid sequences of HCS (HPL), HGH, and Prolactin (Pr). The first fourty amino acids of the prolactin sequence are those of human prolactin. Homologous sections of the remaining amino acids are derived from the sequence of ovine prolactin. Homology is indicated as follows: Identical pairs by a vertical bar, highly acceptable pairs by three dots, and acceptable pairs by a single dot. Nonhomologous pairs are indicated by a cross.

(From: Textbook of Endocrinology, Ed. Robert H. Williams, Page 47, Fifth Edition, 1974, W.B. Saunders Company, Philadelphia, U.S.A. With Permission)

as two of the three ones in human and ovine prolactin (Li, 1972; Niall, 1972; Lewis et al., 1972). In Fig. 1 the primary structure of these hormones are compared to each other.

Radioimmunoassays were available for rat prolactin (Kwa et al., 1969). Because of the noticed cross-reaction with ovine prolactin, heterologous assays for human prolactin were developed using antisera raised against ovine prolactin. After a short time, however, sufficient amounts of human prolactin were purified to raise antisera against it to develop highly sensitive homologous radioimmunoassays (Guyda et al., 1971; Hwang et al., 1971; Lewis et al., 1971 (b); Friesen et al., 1972 (a); and Sinha et al., 1973). All these assays gave comparable results in terms of physiopathological findings in man, but because of the large variations in the absolute values, it may be that the antibodies bind to different immunological sites of the prolactin molecule. As in all radioimmunoassays, there is a need for international standardization. At this moment, different groups are discussing these problems to find a suitable solution.

During the last few years an extremely large amount of information has been published concerning the physiopathology of human prolactin because of the availability of the radioimmunoassays mentioned. Many of the postulated functions in the human being are still hypothetical and only those will be mentioned which definitely seem to be of some importance (Compare Table 1).

## Central Regulation

Prolactin, like other protein hormones, is produced in the anterior pituitary gland under control of the hypothalamus. In contrast with the gonadotrophic hormones, prolactin release is controlled by a **P**rolactin **I**nhibiting **F**actor (PIF) produced within the hypothalamus. This factor has not as yet been isolated, but an increase of the dopamine concentration in the neurons of the hypothalamus decreases the release of prolactin from the pituitary. The presumption was that dopamine increased the amount of PIF within the hypothalamus.

Dopamine itself is not able to cross the blood-brain barrier. L-dopa, however, does and is converted into dopamine within the hypothalamus. Administration of this drug indeed lowered peripheral plasma levels of prolactin in women with galactorrhea (Malarkey et al., 1971). Recent studies have been undertaken in vitro, demonstrating a direct inhibitory effect of dopamine itself at the pituitary level which can be inhibited in a dose-dependent way using drugs interfering with its action. Therefore, at the present time, it seems reasonable to believe that dopamine itself is PIF which, once released within the hypothalamus, will be transported via the venous system to the pituitary where it exerts its action (Smalstig and Clemens, 1974; Takahara et al., 1974; Dibbet et al., 1974). In the case of hyperprolactinemia, L-dopa administration can help us to differentiate between an autonomous pituitary prolactin producing adenoma or a secondary hyperplasia of the prolactin producing cells due to hypothalamic disorder.

Synthetic **T**hyrotrophin **R**eleasing **H**ormone (TRH) has been found to release prolactin from cultured rat pituitaries (Tashjian et al., 1971). Soon thereafter, in vivo studies in man also showed the same response (Bowers et al., 1971). Many investigators believe that TRH must be the long postulated **P**rolactin **R**eleasing **F**actor (PRF). In fact, administration of TRH and dopamine in vitro studies in rats has shown that the prolactin response to TRH can be modulated by concomitant administration of dopamine without changing the response of TSH to TRH (Takahara et al., 1974; Dibbet et al., 1974). In the case of hypothalamic-hypophyseal dysfunction, administration of TRH can be used to investigate pituitary function.

Oestrogens are known to stimulate prolactin secretion in animals (Meites, 1969). This effect in man seems less clear. L'Hermite and co-workers (1972) reported an increase of prolactin in postmenopausal women treated with high dosages of oestrogens with advanced breast cancer. Prolactin increase has also been reported in male patients during oestrogen treatment (Frantz et al., 1972 (b)). The increase of prolactin during pregnancy in humans parallels the increase of oestrogens. In the monkey there is no prolactin increase until just before parturition. Because in monkey pregnancy the oestrogen metabolism is different compared to humans, this could explain the difference in prolactin metabolism. Oestrogens seem to have an indirect function via the hypothalamus to stimulate prolactin production in the pituitary and a direct influence on the hypophysis to stimulate release (Horrobin, 1973 (c)).

A large scale of different psychotrophic drugs have been reported to stimulate prolactin release by acting indirectly on the hypothalamus (For reviews see: Sulman, 1970 (b); Verhoeven, 1974). The phenothiazines are of special interest because of the frequent use and the clinical importance of these drugs. Their action, as known so far, seems to be a depletion of catecholamines within different areas of the brain reducing the PIF content in the hypothalamus and, thus, inducing prolactin release. In the case of hypothalamic-hypophyseal dysfunction, administration of chlorpromazine has become a useful test in examining responsiveness of this axis (L'Hermite, 1973).

Insulin induced hypoglycemia induces prolactin release (Frantz and Kleinberg, 1970). A dose of 0.2 units of insulin per kg must be given to obtain a constant response (Frantz et al., 1972 (b)). The administration of insulin has to give a quick drop in the blood glucose levels, otherwise the prolactin response is blunted. The action is thought to be via the hypothalamus and this response can be used to measure the hypothalamic-pituitary axis (Copinschi et al., 1972).

Ergot-alkaloids, especially 2-Bromo-$\alpha$-ergocryptine (Bromergocryptine, CB 154, Sandoz, Basel, Switzerland) can inhibit prolactin secretion due to a direct action on the prolactin producing pituitary cells. The clinical importance of this drug seems to be great and in the different articles presented in this thesis more information will be given concerning this drug.

Combining the effect in the different drugs mentioned so far (TRH, L-dopa, insulin, chlorpromazine and Bromergocryptine) and because of the availability to measure prolactin by radioimmunoassay, hypothalamic-hypophyseal (dys)function can be examined very carefully. It must be stated that because of the changing values of prolactin in plasma by a diurnal rhythm and by an intermittent release, isolated samples do not give accurate information. If possible, pharmacodynamic tests should be undertaken as described by L'Hermite (1973) before conclusions can be drawn whether this axis is intact or not.

**Normal blood levels**

Many studies have been reported dealing with normal values in males, females, and children. Among the different investigators, slight differences among the absolute values are found. This can be explained due to the use of different standards and especially to intrinsic differences related to the type of assay. The mean level in adult males is about 5.0 ng/ml plasma (range: 0-28 ng/ml), in adult females 8.1 ng/ml plasma (range: 0-28 ng/ml), and in children 10.8 ng/ml plasma (range: 7-17 ng/ml) (Horrobin (d), 1973). Friesen et al., (1972 b) reported no statistically significant difference between children and adults. The mean value for female compared to male is slightly higher. It is not yet established whether this sex linked difference is statistically significant or not.

**24 hour secretion pattern**

Both in male and in female, prolactin demonstrates a circadian rhythm with maximum serum prolactin levels between 1 and 5 a.m. (Nokin et al., 1972) During the morning these levels decrease to a minimum between 10 a.m. and 12 noon. This rhythm is not necessarily related to the cycle of sleeping and waking and the physiological importance is not yet understood (Vanhaelst et al., 1973).

**Menstrual cycle**

The reports on changes in plasma prolactin levels during the human menstrual cycle have been conflicting. L'Hermite and co-workers reported a small prolactin peak at midcycle concomitant with the preovulatory oestrogen peak, and higher levels of prolactin during the luteal than the follicular phase of the cycle (L'Hermite et al., 1972; Robyn et al., 1973). This increase fits well with the increase of oestrogens at the same time. Other groups have failed to demonstrate this (Hwang et al., 1971; Friesen et al., 1972 (a)), although higher levels were found in some women during the luteal phase (McNeilly and Chard, 1974). It is my opinion that this should be examined more carefully by drawing frequent blood samples throughout the days around the ovulation or by estimating prolactin in urine during the cycle. Also we have to keep in mind that until now, all reports have dealth with plasma levels of this hormone. However, this gives no information about the tissue concentration of the hormone or the amount of hormone receptors at a given time which can depend on factors other than only the release of prolactin from the pituitary gland.

In conditions with galactorrhea and menstrual disturbances, the role of increased plasma prolactin concentration on reproductive endocrinology is obvious. These conditions will be discussed more in detail in the second article of this thesis.

**Pregnancy**

From the onset of the human pregnancy there is a steady increase in plasma prolactin levels, reaching a maximum during the third trimester of about 200 ng/ml plasma (Tyson et al., 1972). As mentioned earlier, this increase does not take place in monkeys, and the difference between these two species could be due to the nearly complete lack of oestrogens in pregnant monkeys.

Surprisingly, however, both human and monkey amniotic fluid were found to contain large amounts of immunoreactive prolactin indistinguishable from pituitary prolactin. The maximum concentration occurs around midpregnancy with a mean of approximately 4000 ng/ml amniotic fluid with a slight decline thereafter until term. There is little transfer of the hormone from the amniotic fluid into the foetus, or from the mother into the amniotic fluid. According to present findings, the chorion seems capable of producing prolactin. The function of this high hormone concentration is unknown. However, osmoregulation seems a possible explanation (Friesen et al., 1973).

The role of prolactin on lactation and the plasma levels of prolactin during the postpartum period will be discussed in the first, third and fourth article in this thesis.

**Osmoregulation**

Administration of ovine prolactin to normal subjects has been shown to decrease renal excretion of sodium, potassium, and water with a slight increase in plasma sodium concentration and osmolarity (Horrobin, (e), 1973). Other groups have reported a slight increase of plasma prolactin levels in patients with advanced renal failure (Friesen et al., 1972 (a); Frantz et al., 1972 (b)). In a recent report it was shown that in patients with hyperprolactinemia, a waterload did not decrease plasma prolactin levels as in normal controls. A delayed excretion of this waterload and lack of an adequate solute excretion in the hyperprolactinemic subjects were found to support the concept that prolactin plays a significant role in renal handling of water and solute (Buckman et al., 1974). We have to keep in mind, however, that in patients with elevated prolactin secretion, steroid production in the gonads seems to be decreased. Comparing these patients to normal controls, therefore, can not give exact information about prolactin and renal function (Thorner et al., 1974; Article two, three and four, this thesis). The vascular changes during pregnancy with the tendency of increased water and sodium retention could be due to the increasing amount of prolactin throughout pregnancy. Studies have to be undertaken to identify this mechanism.

**Prolactin and thyroid function**

The precise relationship between prolactin and thyroid function is not yet known. The previously reported finding that TRH releases both TSH and prolactin led to the question whether prolactin could play a role in the physiological control of the thyroid gland. This does not seem to be the case. It might, however, give an explanation for the clinical pictures of thyroid dysfunction, galactorrhea and menstrual disturbances.

In hypothyroidism due to thyroid failure, an increase of TSH can be measured as a reaction to the lack of feedback mechanism of thyroxine on TRH release. In these cases elevated prolactin levels sometimes are found together with galactorrhea and oligo- or amenorrhea which completely cured after thyroxine replacement therapy (Kinch et al., 1969; Edwards et al., 1971). That the prolactin did not completely correlate with TSH can be explained by keeping in mind that prolactin secretion next to TRH also is controlled by an inhibitory mechanism not affecting the TSH release.

In humans, there also have been some reports dealing with the rare syndrome of prepubertal primary hypothyroidism, galactorrhea and precocious puberty (Van Wyck and Grumbach, 1969). In these children the combination of hypothyroidism and galactorrhea can be explained by lack of thyroxine to inhibit TRH secretion. Elevated prolactin levels have been measured and thyroxine replacement reduced these levels to normal with cessation of the galactorrhea (Horrobin (f), 1973). To give an explanation for the precocious puberty is rather difficult. As will be shown later, prolactin is thought to decrease the sensitivity of the ovaries to gonadotrophins in adults. Recently, it has been shown that gonadotrophins (FSH and LH) and TSH all consist of two peptide subunits, namely the $\alpha$- and $\beta$-chain. The $\alpha$-chain seems to be nearly similar in all three hormones, and between TSH and LH there is also an overlap of at least 50% in the $\beta$-chain. It is possible that in children the increased amount of TSH itself could have some luteotrophic effect, but the pituitary gland could also release incomplete TSH with increased luteotrophic effect due to the continuous hyperactivity because of the elevated TRH: This luteotrophic effect would then overcome the role of prolactin (Daughaday, 1974).

In the case of hyperthyroidism, the same picture could be expected if the disease was due to hypothalamic overproduction of TRH. Patients have indeed been reported with this disease combined with galactorrhea and hyperoestrogenic menstrual disturbances (Zondek et al., 1951).

## Prolactin and human breast cancer

At least in the rat it has been established that prolactin and oestrogens are the principal hormones involved both in breast tumor growth and regression (Pearson et al., 1969; McGuire et al., 1974). In man, however, there exists a controversy about this mechanism. Both surgical hypophysectomy, which abolishes prolactin secretion, and pituitary stalk section, which mainly results in increased prolactin secretion, can produce regression of tumor growth in breast cancer patients (L'Hermite, 1973).

Measurement of prolactin concentrations in patients with breast cancer and without did not give a significant difference in these concentrations (Wilson et al., 1973; Boyns et al., 1973; Kwa et al., 1974). When plasma levels of prolactin from members of breast cancer families (high-risk group) were compared to the levels in breast cancer patients and controls, the prolactin levels in the high risk group were significantly higher than in the others (Kwa et al., 1974). This could indicate a role of prolactin in the development of the tumor rather than in maintenance of tumor growth once the tumor has developed. Also in rats which respond to 7,12-dimethylbenz ($\alpha$) anthracene (D.M.B.A.) treatment with breast cancer development, there was a correlation between plasma prolactin levels and the genetically determined susceptibility to breast cancer (Wilson et al., 1973).

At the present time radioreceptorassays for prolactin have been developed showing binding of prolactin to mammary cell membrane-suspensions (Shiu et al., 1973). McGuire and co-workers demonstrated prolactin receptors in rat breast cancer tissue (Costlow et al., 1974). Culture studies using tissue from human breast carcinomas are at the moment being undertaken to establish whether these are prolactin-dependent or not (McGuire et al., 1974). Using the new technique mentioned above, it is my personal opinion that the role of prolactin on both breast cancer development and growth will be more fully understood in the near future.

## Experimental models of this thesis

The influence of prolactin on different endocrine functions in the human female can be studied in vivo comparing hormonal data of patients with hyperprolactinemia or lactating women to normal, nonlactating controls. Using the effect of Bromergocryptine, however, patients can be followed before and during administration of this drug. This allows us to observe clinical and hormonal changes within one and the same subject as the amount of prolactin decreases back to normal. Another possibility is to compare lactating and non-lactating, Bromergocryptine treated women to each other during the early part of the puerperium at which time prolactin levels are known to be elevated (Tyson et al., 1972).

The first article of this thesis deals with the effect of Bromergocryptine on puerperal lactation. The three following papers give information on the influence of prolactin on ovarian function in the human female using the two experimental models as mentioned above.

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# A NEW APPROACH TO THE INHIBITION OF PUERPERAL LACTATION

BY

RUNE ROLLAND

AND

LOUIS SCHELLEKENS

*Department of Gynaecology and Obstetrics, The "Wever Hospital", Heerlen, The Netherlands*

**Summary**

Inhibition of lactation by the new peptide ergot alkaloid 2-Br-α-ergocryptine (CB154) was studied in a double-blind trial Forty patients divided into four groups were treated during seven consecutive days with daily doses of either 2 5 mg , 5·0 mg , 7 5 mg of CB154 or a placebo In terms of milk secretion, engorgement and pain the CB154 therapy was significantly better than the placebo Tolerance of CB154 was good and no drug-related side effects were observed In a further trial CB154 was tried in five patients with already established lactation and in 22 patients who had not responded to standard hormonal therapy With one exception (suppression of lactation after four months of full breast-feeding) excellent results were achieved

For prevention as well as for the suppression of already established lactation, the following dosage schedule of CB154 is effective and avoids a rebound phenomenon: 2 5 mg twice daily for two weeks, followed by 2 5 mg once daily for one week

UNTIL recently, the inhibition of lactation has been attempted by one or more of the following methods. (a) hormonal inhibition by oestrogens alone, or in combination with androgens; (b) mechanical compression of the breasts; (c) restriction of fluid intake, supplemented by diuretic agents

Treatment with oestrogens, in combination with mechanical compression of the breasts, has been most effective with a failure rate of 10 to 20 per cent, the same number of patients develop late mammary congestion after 10 to 14 days (Daniel *et al*, 1967; Hodge, 1967) For certain groups of patients this method of inhibition enhances the risk of thromboembolic complications in the puerperium (Daniel *et al*, 1967, Jeffcoate *et al*, 1968). The third method, used alone, is ineffective

Recently a new peptide ergot alkaloid, 2-Br-α-ergocryptine, has been developed, which reduces the serum-levels of prolactin (Besser *et al*, 1972; Del Pozo *et al*, 1973). CB154 (Sandoz, Basle) is 2-Br-α-ergocryptine methane sulfonate, a crystalline powder of yellowish-white colour. The molecular weight of the free base is 654·5, that of the salt 750 6. The struc-

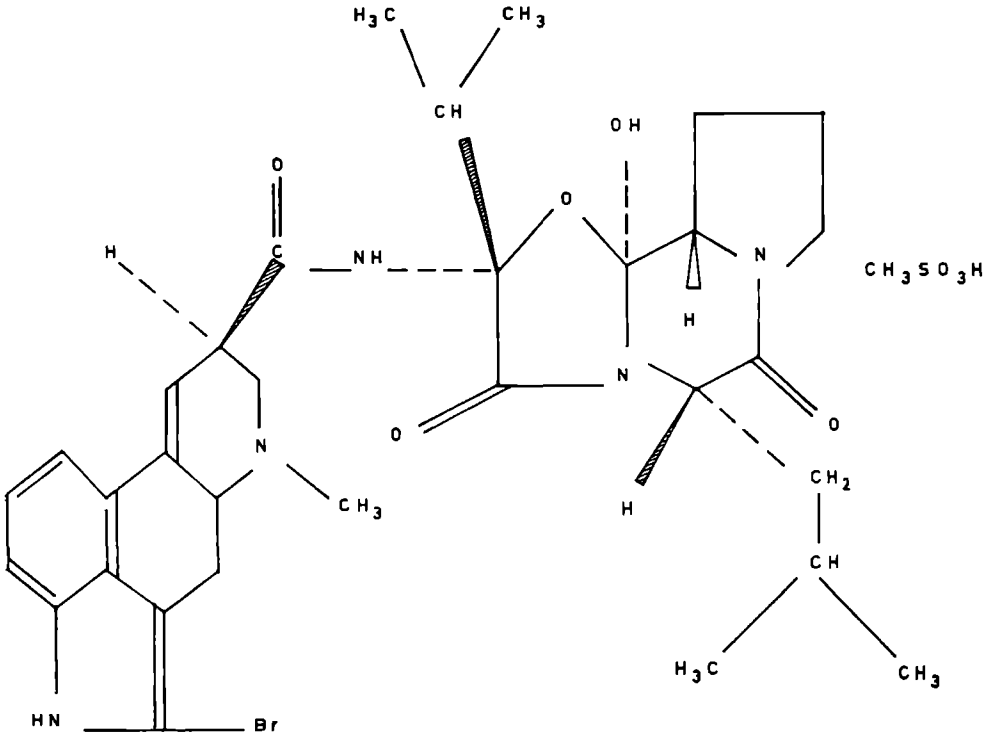


FIG. 1
Structure of CB154.

ture of this preparation is shown in Figure 1. CB154 probably acts at the level of the pituitary gland (Del Pozo *et al.*, 1973; Pasteels *et al.*, 1971). The suppressive effect on lactation has been successfully proved in several animal studies (Billeter and Flückiger, 1971; Flückiger and Wagner, 1968) and also in puerperal women (Varga *et al.*, 1972). In the same way, CB154 is active in women with galactorrhea and disturbances of the menstrual cycle (Lutterbeck *et al.*, 1971; Besser *et al.*, 1972; Leutenegger *et al.*, 1972; Varga *et al.*, 1973) and in men with galactorrhea and impotence (Besser *et al.*, 1972). We report here the effect of CB154 on puerperal lactation and define effective dosage schedules.

## PATIENTS AND METHODS

In a double blind trial using capsules of identical appearance, daily doses of 2·5 mg., 5·0 mg. and 7·5 mg. of CB154 were compared with a placebo. Forty women who had given proof of sufficient lactation in a previous puerperium were studied, in four groups of 10, for 17 days. For the first seven days each patient received at random a package of 21 capsules, one to be taken three times a day before meals. The first capsule was always given within two to six hours following delivery, and no other specific treatment was given. A summary of the distribution of the daily medication to these four groups is shown in Table I. In addition oxytocics, hypnotics or iron preparations were

TABLE I

*Dosage schedule for the 7 days of treatment*

| Time | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| 0800 hours | 2·5 mg. | 2·5 mg. | 2·5 mg. | Placebo |
| 1300 hours | 2·5 mg. | 2·5 mg. | Placebo | Placebo |
| 1900 hours | 2·5 mg. | Placebo | Placebo | Placebo |
| Total dose of CB154 per day | 7·5 mg. | 5·0 mg. | 2·5 mg. | None |

given in some cases. The effectiveness of treatment with CB154 was assessed three times daily by recording milk secretion, engorgement of the breasts and complaints of pain, according to the rating scale shown in Table II. On the 7th day postpartum, when leaving the hospital, both the patients and the responsible doctor had to decide whether or not they were satisfied with the treatment so far; for the following ten days the patients were asked to record symptoms of mammary activity and on the 17th day they returned a questionnaire on the results of treatment.

The four groups were statistically identical in terms of age, body-weight, duration of pregnancy and parity. Patients on medication which might influence their hormonal state and patients with hypertension (diastolic pressure more than 100 mm. Hg) were excluded. If the medication had to be discontinued within the first 72 hours after delivery, the patient in question was excluded and another patient with the same dosage schedule was admitted to the study. The code of the group was not known to the investigators before the study had been concluded.

Possible side-effects of the medication were checked by daily measurement of blood pressure, pulse frequency and diuresis. Before and after the treatment, a full blood count was done and hepatic and renal function tests were performed.

Statistical analysis of breast engorgement, pain, and the overall impression of the success of treatment on the 7th day, was made by the 2 I technique (Kullback *et al.*, 1962). Blood pressure and pulse frequency were analyzed by using the Wilcoxon test and the H test, the diuresis only by the H test. All other parameters could be assessed by analysis of variance.

TABLE II

*Determination of mammary activity (rating scale)*

| Score | Milk production (by palpation) | Congestion | Pain |
|---|---|---|---|
| 0 | No milk | Absent | Absent |
| 1 | Some drops | Mild | Mild |
| 2 | Slight outflow | Moderate | Moderate |
| 3 | Stream of milk | Severe | Severe |

## RESULTS

There was a very clear difference in the success of treatment between the placebo group and each of the CB154 groups regarding the overall clinical impression ($P<0·001$) as shown in Table III. However, there was no statistically significant difference between the three groups treated with CB154.

*Inhibition of milk secretion.* This was excellent in all groups treated with CB154. There was a statistically significant difference between the

TABLE III

*Overall clinical impression of the effect of treatment by physician and by patients*

| Group | Number of patients | | | |
|---|---|---|---|---|
| | Good effect | | Poor or no effect | |
| | assessment by physician | assessment by patient | assessment by physician | assessment by patient |
| CB154 2·5 mg. daily | 8 | 8 | 2 | 2 |
| CB154 5·0 mg. daily | 10 | 10 | 0 | 0 |
| CB154 7·5 mg. daily | 10 | 10 | 0 | 0 |
| Placebo* | 0 | 1 | 10 | 9 |

* Treatment stopped after four days by eight of ten patients.

TABLE IV

*Inhibition of milk secretion*

(Mean scores, see Table II)

| Groups | Mean scores on treatment days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 2·5 mg. of CB154 daily | 0·50 | 0·53 | 0·53 | 0·50 | 0·71 | 0·48* | 0·52* |
| 5·0 mg. of CB154 daily | 0·13 | 0·37 | 0·57 | 0·43 | 0·27 | 0·23 | 0·13 |
| 7·5 mg of CB154 daily | 0·50 | 0·73 | 0·57 | 0·53 | 0·50 | 0·27 | 0·27 |
| Placebo | 0·33 | 1·00 | 0·66 | 1·50** | *** | *** | *** |

* N = 9; increase of dosage in one case.
** N = 8; interruption of placebo treatment in two cases.
*** N = 2; treatment continued in two cases only.
\+ = P<0·05.
\+ + = P<0·01.
\+ + + P<0·001.

treated groups and the placebo group (P<0·01 for the 2·5 mg. and 5·0 mg. daily dosages and P<0·05 for the 7·5 mg. daily dosage). When the three treatment groups are combined, the results are highly significantly better than the placebo group (P<0·001); between the three CB154 schedules no statistical difference was found.

For this analysis the results from the fourth day were used, because in the placebo group treatment had to be stopped by eight of the ten patients because of mammary activity developing within 90 hours of delivery (Table IV; Fig. 2).

*Prevention of mammary engorgement.* This was effective in all CB154 treated groups. On the other hand mammary engorgement appeared to a statistically significant degree more frequently in the placebo group than in the others (P<0·001 for the groups treated with 5·0 mg. and 7·5 mg. daily and P<0·05 for the group treated with 2·5 mg. daily). There was also a clear difference between the 2·5 mg. group compared to the two others (P<0·01), but there was no difference between the 5·0 mg. and the 7·5 mg. treated groups (Table V).

*Complaints of pain.* These were much more frequent in the placebo group, with a statistically highly significant difference from the other three groups (P<0·001). On the other hand between the three CB154 treated groups no statistical difference was shown (Table VI). When noticed,

TABLE V

*Complaints of mammary engorgement during the first seven treatment days*

| | Present | Not present |
|---|---|---|
| CB154 2·5 mg daily | 4 | 6 |
| CB154 5·0 mg. daily | 0 | 10 |
| CB154 7·5 mg daily | 0 | 10 |
| Placebo* | 9 | 1 |

* Treatment stopped after four days by eight of ten patients.
\+ = P<0·05.
\+ + = P<0·01.
\+ + + = P<0·001.

TABLE VI

*Complaints of pain during the first seven treatment days*

| | Present | Not present |
|---|---|---|
| CB154 2·5 mg. daily | 3 | 7 |
| CB154 5·0 mg. daily | 1 | 9 |
| CB154 7·5 mg. daily | 2 | 8 |
| Placebo* | 9 | 1 |

* Treatment stopped after four days by eight of ten patients.
\+ = P<0·05.
\+ + = P<0·01.
\+ + + = P<0·001.

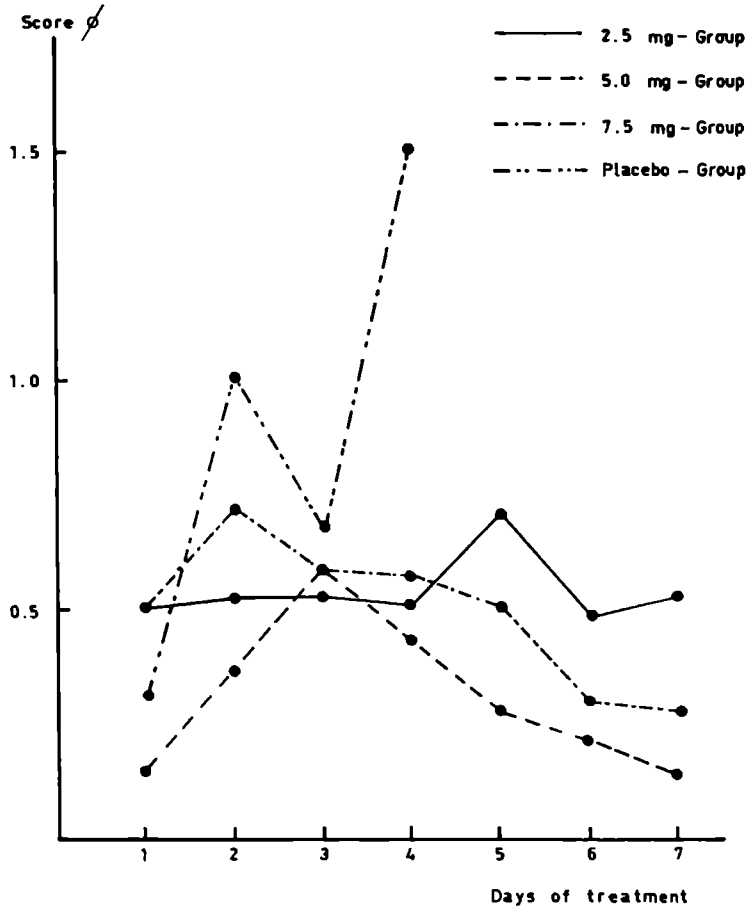


FIG. 2
Mammary secretion.

the complaints were mild or sometimes moderate but of short duration in the treated groups as opposed to the placebo group where the complaints in nine of ten patients became severe within 90 hours of delivery.

*Follow-up.* Seven days of CB154 therapy was not sufficient in all patients to prevent lactation; a rebound phenomenon was seen in 21 patients within 72 hours of leaving hospital. In a further two patients the daily dose of 2·5 mg. was not high enough and had to be increased to 5·0 mg. in one case on the 6th and in the other on the 8th treatment day. After three weeks of CB154 medication the therapeutic effect was satisfactory. Only seven patients had no complaints at all, three in the 2·5 mg. group, one in the 5·0 mg. group and three in the 7·5 group. Of the 21 women with rebound symptoms two were lost to further follow-up and another five were treated with hormones by their general practitioner; three of these five women showed a very slow recovery from their symptoms with

much discomfort. The remaining 14 patients received a second course of treatment with CB154, being given 2·5 mg. twice daily for the first week, followed by 2·5 mg. once daily for the next week. Approximately one hour after starting the treatment the breasts had to be emptied mechanically in 11 cases because of excessive discomfort, but after 24 hours of medication all 14 patients were nearly free from symptoms and no further rebound phenomena were seen when treatment was discontinued two weeks later.

*Placebo group.* Two patients must be mentioned separately. One of them had clear evidence of engorgement and milk secretion on the fourth and fifth days, but there was a very quick decline of her symptoms and she was discharged from hospital with no sign of mammary activity. The second patient showed mild to moderate symptoms of initial lactation from the third day and, when leaving the hospital, she was given a course of treatment as mentioned above, with the same good result. The other eight had to be considered as failures of therapy, and treatment was stopped within 90 hours after delivery. With seven of these eight patients we tried a delayed suppression of lactation by CB154 with the following dosage-schedule: 2·5 mg. thrice daily for the remaining three or four days in hospital, followed by one week of 2·5 mg. twice daily and one week of 2·5 mg. daily. The breasts were always emptied mechanically approximately one hour after commencing treatment. Just as in the patients with a rebound phenomenon, the complaints disappeared almost completely within 24 hours, with no further signs of mammary activity after ceasing the medication.

No statistically significant influence of CB154 was demonstrated on blood pressure, pulse frequency, urinary output or the specific gravity of urine. All the laboratory examinations were within normal limits before and after the study. No side effects occurred as a result of CB154 medication; various mild complaints such as nausea and giddiness were noted just as frequently in the placebo group as in the treatment groups.

## Discussion

In our opinion CB154 demonstrated a better lactation inhibiting effect than the combination of hormonal and mechanical treatment previously used in our clinic. In particular the almost complete relief of pain and engorgement spared both the patient and the nursing staff many complaints, and justified the longer duration of treatment. The following dosage schedule seems to be optimal: starting within 24 hours of delivery for the first two weeks 2·5 mg. twice daily, followed by 2·5 mg. once a day for the third week. No further treatment is necessary. It was interesting that in the placebo group signs of mammary activity did not really become apparent until 40 to 72 hours after delivery.

After completing this study in June 1972, we treated 22 patients, for whom hormonal suppression had failed, with CB154, using the dosage schedule mentioned above, with the same excellent results. As CB154 acts by suppressing the release of prolactin from the pituitary gland, this supports the thesis that the steroid hormones act by some other mechanism, probably in the mammary gland itself (Tyson *et al.*, 1972).

In the same period we also used CB154 to suppress lactation in five mothers who needed this for various reasons after establishment of full mammary activity. With the same dosage schedule we had very good results in four patients; in one of them, treatment was only started 20 days postpartum. The fifth patient was our only failure of therapy: she had been breast feeding for four months, but because of mammary infection lactation had to be reduced. Three months after delivery her menstrual cycle was re-established. We had no success with a dosage of 7·5 mg. of CB154 daily. This might suggest another mechanism of regulation of physiological lactation, independent of prolactin, after re-establishment of the menstrual cycle. In patients with persistent prolactin-dependent galactorrhea, mostly in combination with amenorrhea or non-ovulatory oligomenorrhea, a quick cessation of milk production is seen during CB154 medication with restoration of ovarian function (Lutterbeck *et al.*, 1971; Besser *et al.*, 1972; Varga *et al.*, 1973). Prolactin might suppress release of FSH and LH or inhibit the response of the ovaries to these hormones. Normal levels of gonadotrophins or of oestrogens and progesterone could in their turn inhibit the further release of prolactin (Reyes

*et al*, 1972, Zarate *et al*, 1972) To clarify this mechanism a further study is now being undertaken, comparing puerperal women whose lactation has been suppressed by CB154 with puerperal lactating women, an earlier restoration of ovarian function is expected in women treated with CB154

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# SUCCESSFUL TREATMENT OF GALACTORRHOEA AND AMENORRHOEA AND SUBSEQUENT RESTORATION OF OVARIAN FUNCTION BY A NEW ERGOT ALKALOID 2-BROM-α-ERGOCRYPTINE

RUNE ROLLAND, LOUIS A. SCHELLEKENS AND RUDOLF M. LEQUIN*

*Department of Obstetrics and Gynaecology, 'De Wever' Hospital, Heerlen, and*
**Department of Obstetrics and Gynaecology, University Hospital, Nijmegen, The Netherlands*

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SUMMARY

Three patients with galactorrhoea and menstrual disturbances, due to hyperprolactinaemia and of more than 2 years duration, are described. Administration of 2-Brom-α-ergocryptine effectively lowered the plasma prolactin concentrations in all three patients and restored the normal cyclic ovarian activity. One pregnancy was noticed during therapy. The role of prolactin on ovarian function is discussed. 2-Brom-α-ergocryptine seems to be an effective drug in the long-term treatment of galactorrhoea associated with menstrual disturbances.

Abnormal lactation in combination with menstrual disturbances has until recently been regarded as a rather uncommon syndrome (Canfield & Bates, 1965; Thompson & Kempers, 1965; Rankin *et al.*, 1969). Owing to a growing world-wide interest in the mechanism regulating the hypothalamic–hypophyseal–ovarian axis, and because of the development of sensitive radioimmunoassays for FSH and LH as well as human prolactin (Midgley, 1966, 1967; Hwang *et al.*, 1971), the syndrome of galactorrhoea and amenorrhoea has attracted increasing interest. In the last few years a number of papers have been published concerning this matter (Besser & Edwards, 1972; Besser *et al.*, 1972; Turkington, 1972; Zárate *et al.*, 1973). We traditionally distinguish between the Chiari-Frommel syndrome consisting of galactorrhoea and amenorrhoea following a presumably normal parturition (Chiari *et al.*, 1958; Frommel, 1958). Galactorrhoea with amenorrhoea, usually occurring in nulliparous women, is classified as the syndrome of Argonz–del Castillo (Argonz & del Castillo, 1953). In association with pituitary enlargement we speak of the Forbes–Albright's syndrome (Forbes *et al.*, 1954). The primary cause of the development of these syndromes is not yet known. Patients have been reported evolving through the various syndromes with the additional manifestation at a later stage of Cushing's disease due to chromophobe adenomas of the pituitary gland (Young *et al.*, 1967; Mahesh *et al.*, 1969).

Correspondence: Dr R. Rolland, Department of Obstetrics and Gynaecology, 'De Wever' Hospital, Heerlen, The Netherlands.

In general, however, hypothalamic dysfunction seems to be an acceptable hypothesis. This could lead to enlargement of the pituitary gland as a reaction to the continuous stimulus to produce prolactin. It is well known that during pregnancy the pituitary increases in volume with concomitantly an increased prolactin production (Käser *et al.*, 1967; Reyes *et al.*, 1972). The common denominator in these examples does indeed seem to be high plasma levels of prolactin. An influence of prolactin on ovarian function is probable, although the exact mechanism of action is uncertain. There are indications that this takes place at the hypothalamic level, but also an antigonadotrophic effect of prolactin *per se* on the ovaries has been postulated (Reyes *et al.*, 1972; Zárate *et al.*, 1972).

2-Brom-α-ergocryptine (bromergocryptine, code name CB 154, (Sandoz, Switzerland)) is a new semi-synthetic tripeptide ergot alkaloid. This drug shows specific antigalactic activity almost free of side effects. This effect has been demonstrated in several experimental animal studies and also in puerperal women and in patients with non-puerperal galactorrhoea (Flückiger & Wagner, 1969; Billeter & Flückiger, 1971; Lutterbeck *et al.*, 1971; Besser *et al.*, 1972; Varga *et al.*, 1972, 1973). For example, our group defined a useful dosage-schedule for inhibition of puerperal lactation and also showed that previously established lactation, due in most cases to failure of hormonal therapy, could be supressed by this drug (Rolland & Schellekens, 1973). The pharmacological action of bromergocryptine is at the level of the pituitary gland and functions by blocking the release of prolactin from the hormone producing cells (Pasteels *et al.*, 1971; Del Pozo *et al.*, 1973). By administration of this drug, the effect on galactorrhoea and the reaction of the hypothalamic–hypophyseal–ovarian axis to declining levels of prolactin, have been studied.

## SUBJECTS AND METHODS

Three patients with galactorrhoea and menstrual disturbances treated with bromergocryptine have been carefully studied. The degree of galactorrhoea is classified by an arbitrary scale of +++/++/+ ranging from spontaneous profuse milk secretion from both breasts to only a few drops obtained by copious palpation. Urinary oestrogens and pregnanediol determinations were performed by standard methods (Eechaute & Demeester, 1965; Brombacher *et al.*, 1968a, b). Total urinary excretion of gonadotrophins was measured by the mouse uterus test as described by van Gilse (1953). During treatment heparinized venous blood samples were frequently collected, centrifuged, and the plasma kept at −20°C until assay. Prolactin was measured by an homologous radioimmunoassay kindly supplied by Dr H. Friesen, Montreal (Hwang *et al.*, 1971). The values are expressed as ng/ml plasma. For the LH assay an anti-HCG serum, kindly provided by Organon, The Netherlands, was selected which showed minimal cross-reaction with FSH. As tracer, highly purified LH was used, prepared according to Closset *et al.* (1972). The FSH determinations were performed by an assay using antiserum Batch 3 adsorbed with HCG provided by the N.P.A. (U.S.A.) Both FSH and LH are expressed in mIU of the 2nd IRP of HMG, generously supplied by the Division of Biological Standards of M.R.C., Holly Hill, England.

*Case I (H.Ha)*

This patient had been under our care because of persistent galactorrhoea and amenorrhoea since the delivery of her first child in June 1970. An attempt to inhibit *post-partum* lactation with oestrogen–testosterone was unsuccessful. During the following 2 years she

also developed persistent frontal headache. Her previous history revealed a normal menarche at 15 years followed by a regular menstrual cycle until 1967 when oligomenorrhoea and later on amenorrhoea without galactorrhoea occurred. Ovulation was induced by injections of human menopausal gonadotrophin and human chorionic gonadotrophin (Organon, The Netherlands) which resulted in the previously mentioned pregnancy.

The physical examination before bromergocryptine treatment showed an obese woman; body weight 78·8 kg, height 168 cm. A constant blood pressure of 150/110 mmHg was measured. The breasts were well developed with heavy milk flow induced merely by gentle palpation. This milk flow also occurred spontaneously during the night. The external genitalia together with the vaginal epithelium were moderately atrophic, showing no signs of oestrogenic activity. The uterus was small, the ovaries could not be palpated. A planigram of the sella turcica and visual field examinations were all reported as normal. The pre-treatment laboratory findings are shown in Table 1. Hypo-gonadotrophic, hypo-oestrogenic

TABLE 1. Initial laboratory studies in three patients with galactorrhoea and menstrual disturbances

| | Case I | Case II | Case III | Normal range in laboratory |
|---|---|---|---|---|
| Total urinary gonadotrophins (MU) (mouse uterus) | 7–15 | 5 | 40 50 | Normal cyclic women: 10–70 MU |
| Urinary total oestrogens/24 hr (μmol) | 0·05 | 0·05 | 0·05–0·12 | Non-pregnant women: 0·04–0·40 μmol/24 hr; 1 μmol = 288 μg (oestriol) |
| Urinary pregnanediol/24 hr (μmol) | 0·2 | 0·6 | 1·6–2·9 | Non-pregnant women in luteal phase: 9–31 μmol/24 hr 1 μmol = 320 μg |
| Urinary 17-ketosteroids/24 hr (μmol) | 37–43 | 39–70 | 24–34 | Normal cyclic women: 25–100 μmol/24 hr; 1 μmol dehydroepi-androsterone = 288 μg. |
| Urinary 17-hydroxysteroids per 24 hr (μmol) | 20–45 | 48–52 | 23–55 | Normal women: 15–55 μmol/24 hr; 1 μmol dehydroepiandrosterone = 288 μg |
| Fasting cortisol plasma level (μmol/1) | 0·40 | 0·41 | 0·69 | Normal women: 0·40–0.70 μmol/1; 1 μmol = 362 μg |
| Thyroid function (TBG, T4) | Normal | Normal | Normal | |
| HGH response to insulin | — | — | Normal | |
| Oral glucose tolerance test | Normal | Normal | Normal | |

amenorrhoea existed, combined with *post-partum* galactorrhoea. All other values determined at this time, fell within normal limits. After collection of some pre-treatment venous blood samples for later determination of prolactin, LH and FSH, treatment was started with 2·5 mg bromergocryptine twice daily during meals. The clinical findings are summarized in Fig. 1. Within 4 weeks of therapy her galactorrhoea had disappeared completely and examination of the uterine cervix revealed very rich mucus showing full signs of oestrogenic activity. Her body temperature curve (BTC) shifted at this time and exactly 2 weeks later she

menstruated for the first time in 3 years. An endometrial biopsy taken on the first day of vaginal bleeding showed a normal menstruating post-ovulatory picture. During the next 13 weeks she had a regular menstrual cycle of 26–28 days with a biphasic basal temperature curve. Galactorrhoea did not recur after lowering the bromergocryptine dose to once daily 2·5 mg. Twenty weeks after starting therapy, amenorrhoea was noticed and a pregnancy test (Organon, The Netherlands) showed HCG activity as in pregnant women. Bromergocryptine medication was immediately stopped. At the moment she is in the last trimester of pregnancy, and routine controls have been so far uneventful.

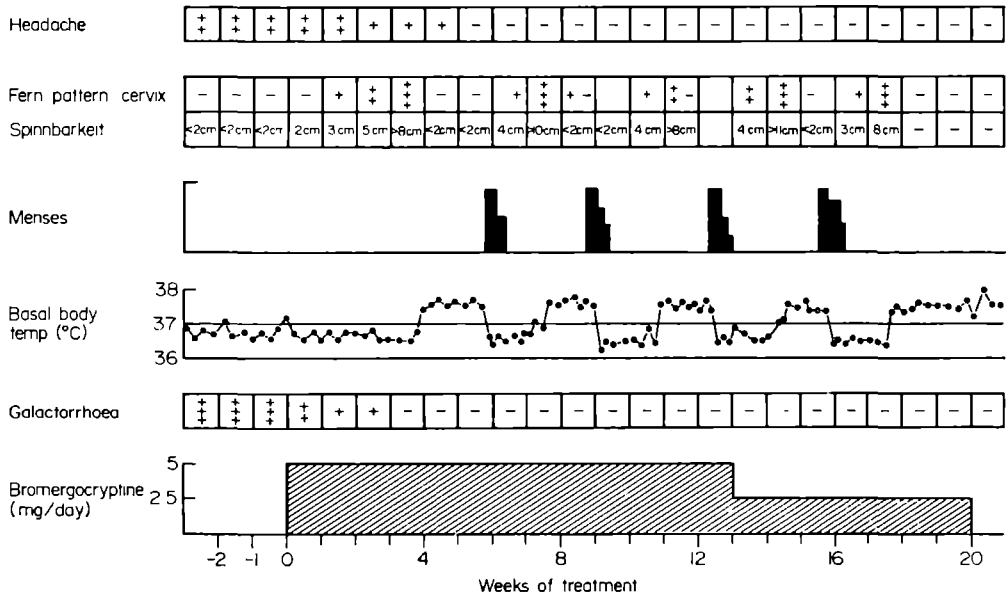


FIG. 1. Case I: clinical findings during bromergocryptine therapy.

The laboratory findings are summarized in Fig. 2. The immediate decrease in the prolactin levels to normal prepregnant values below 30 ng/ml plasma is striking and demonstrates clearly the strong effect of bromergocryptine. The pre-treatment FSH levels are in the high normal cyclic range as we in our laboratory find pre-ovulatory levels of approximately 8 m IU 2nd IRP/ml in normal cyclic women. The LH levels are in the normal cyclic range as established in our laboratory. This is in disagreement with the earlier determined low concentrations of total gonadotrophins in the 24 h urine measured by the mouse uterus test. The amount of oestrogens and pregnanediol are clearly below the levels found in the 24 hr urine of normal cyclic women. As a reaction to the decrease of the plasma prolactin levels, an increase in the amount of excreted total oestrogens is noticable with thereafter an ovulatory LH as well as FSH peak. During the following weeks all levels of measured hormones are in the normal cyclic range with the expected fluctuations. At the end of the treatment period a high level of LH is found; as the patient was pregnant at this moment this must be due to cross-reaction with HCG. It is noteworthy that the FSH levels are undetectable at this time.

*Case II (V.Lu)*

This 22-year-old nulliparous woman had been seen elsewhere because of spontaneous galactorrhoea noticed for the first time in May 1970. At the same time her previous regulatory menstrual cycle became oligomenorrhoeic. Amenorrhoea appeared in January 1972. Induction of ovulation by clomiphene had failed. During the last 2 years she also noticed moderately frontal headache. She married in June 1971, and a pregnancy was wanted.

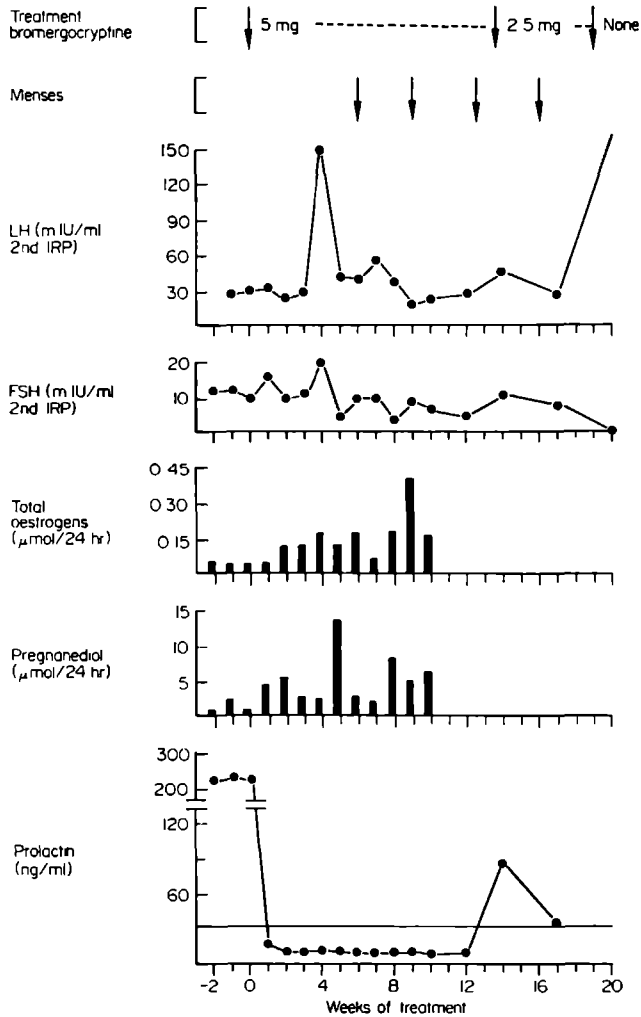


FIG. 2. Case I: laboratory findings during bromergocryptine therapy.

Her previous history revealed a late menarche at 17 years followed by a regular cycle. Physical examination before bromergocryptine treatment showed a healthy young woman with a body length of 161 cm and a body weight of 62 kg. Her hair distribution was female. The breasts were well developed and milk could easily be produced by gentle palpation. This

milkflow also occurred spontaneously, usually during the night. The external genitalia were normal, the vaginal epithelium was atrophic and no signs of oestrogenic activity could be demonstrated in either the vaginal epithelium or in the cloudy mucus of the cervix. The uterus was small, the ovaries could just be palpated. X-rays of the sella turcica and visual field examinations were all reported as normal. The pre-treatment laboratory findings are shown in Table 1. This patient also demonstrates a hypo-gonadotrophic, hypo-oestrogenic amenorrhoea combined with spontaneous but non-puerperal galactorrhoea. Treatment was started with 2·5 mg bromergocryptine twice daily during meals. The clinical findings are summarized in Fig. 3. Her galactorrhoea disappeared completely after 4 weeks of therapy. Examination of the cervix uteri at this time showed the typical aspects of good oestrogenic activity and 1 week later her BTC shifted to a hyperthermic plateau followed by a normal menstruation 12 days later. An endometrial biopsy taken on the first day of vaginal bleeding

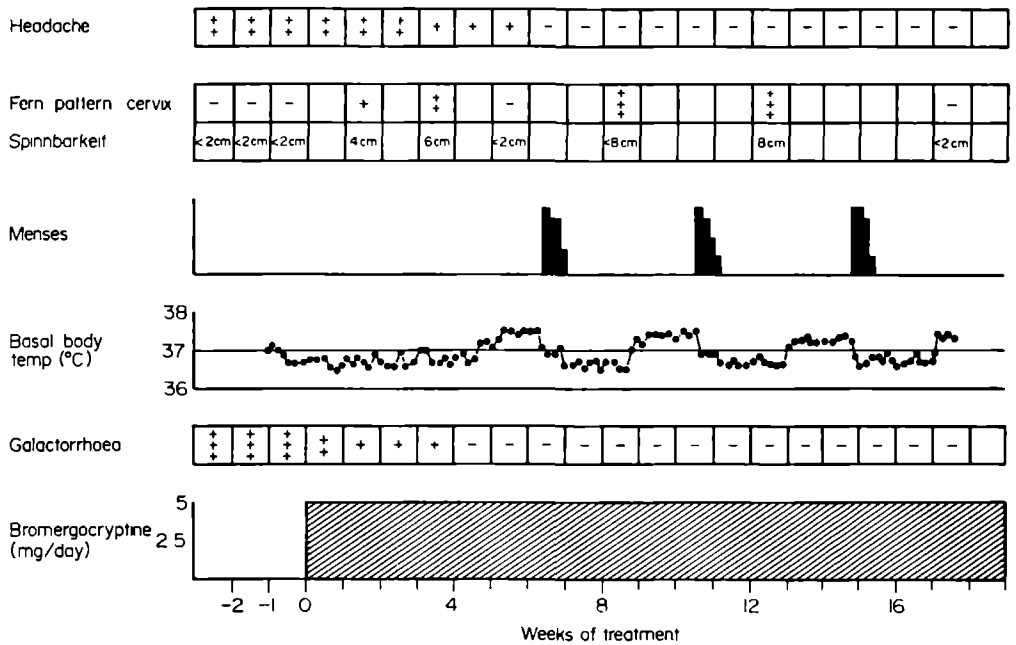


FIG. 3. Case II: clinical findings during bromergocryptine therapy.

showed an endometrial pattern as in the normal menstrual phase. During the next 10 weeks she had a regulatory cycle of 30 days/4 days with a biphasic basal temperature curve. At the moment she is still on therapy without complaints. A pregnancy, however, has not occurred as yet.

The laboratory findings are summarized in Fig. 4. Also here the strong inhibitory effect of bromergocryptine is demonstrated by the rapid decrease of the plasma prolactin levels. The FSH pre-treatment concentrations are in the low cyclic range; the LH levels, however, in the normal cyclic range. These findings are also in slight disagreement with the values of total urinary gonadotrophins previously found by bioassay. During therapy, the 24 hr urinary oestrogen excretion increases to normal values within 4 weeks followed by an increase in

the pregnanediol excretion to values similar to those seen in the normal luteal phase 1 week later. At this time the BTC showed a hyperthermic plateau. Thereafter all hormone values are in the cyclic range with normal fluctuations.

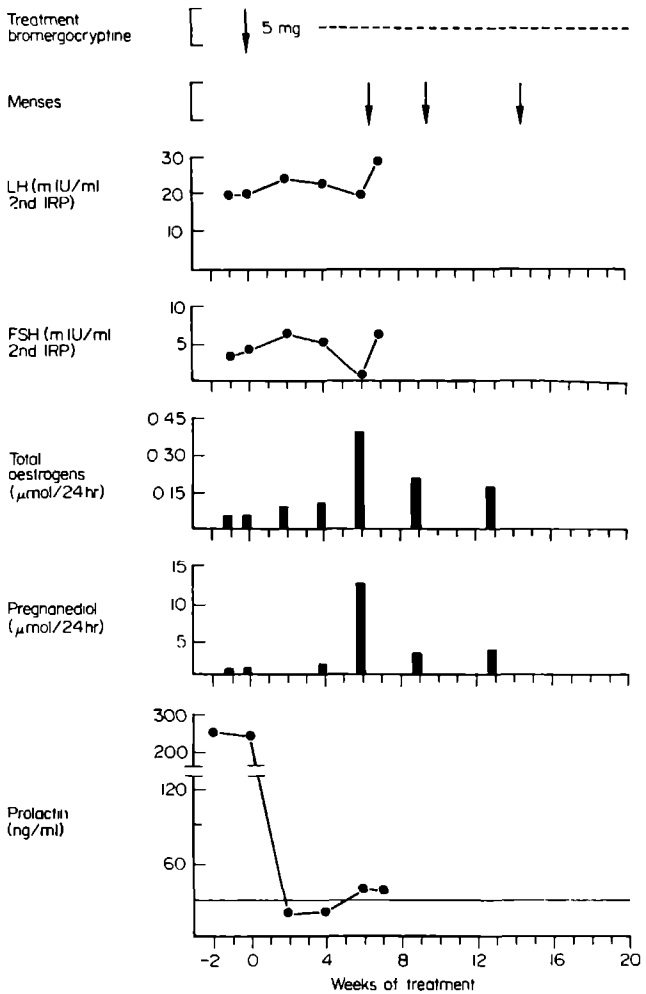


FIG. 4. Case II: laboratory findings during bromergocryptine therapy.

*Case III (G.A.)*

This 27-year-old patient had been under our care since November 1971 because of persistent galactorrhoea and oligomenorrhoea after the birth of her second child in June 1970. In November 1971 amenorrhoea occurred. A microcurettage taken on the first day of her last vaginal bleeding showed endometrium with no signs of secretory activity. During this previous period breakthrough uterine bleeding could sometimes be provoked by progestagen administration. Her menarche was at 13 years followed by a regular menstrual

cycle of 32 days/3 days Just before starting bromergocryptine medication, a spontaneous menstruation was noticed with an endometrial biopsy showing poor secretory activity.

Physical examination revealed a healthy women with female hair distribution. The breasts were well developed with much fibro-glandular tissue. Milk could be easily squeezed out of both breasts by gentle palpation. Her external and internal genital organs were normal with some signs of oestrogenic activity in the vaginal epithelium. Visual field-examinations were normal Because of a doubtful double contour of the bottom of the sella

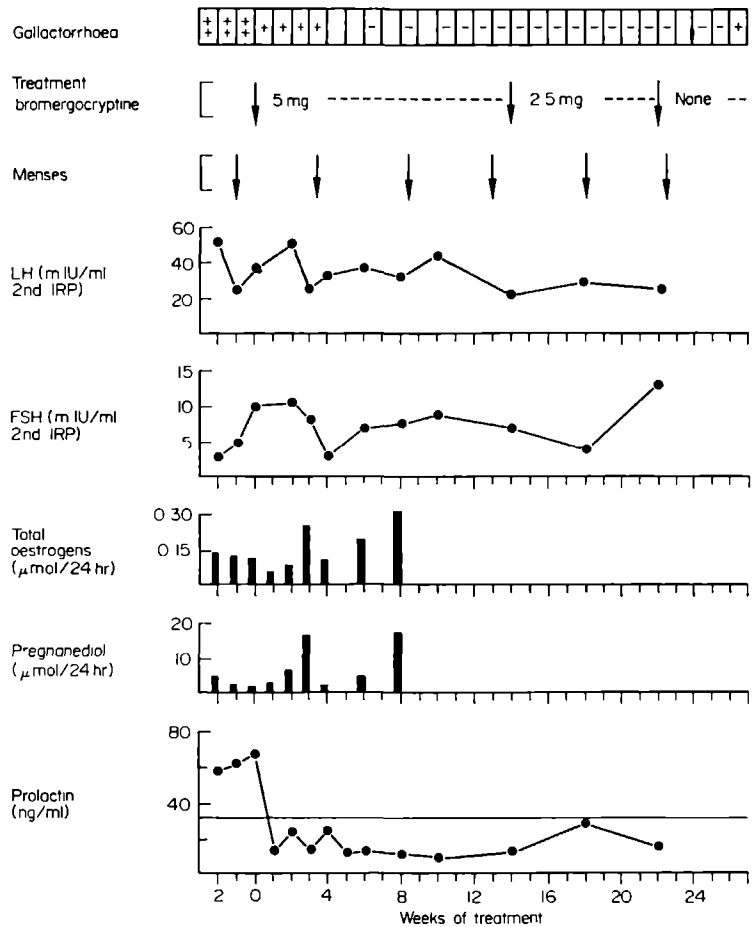


FIG 5. Case III clinical and laboratory findings during bromergocryptine therapy

turcica seen on X-ray, tomography, carotid angiography and cerebral scanning were performed, all with normal results The pre-treatment laboratory findings are shown in Table 1. There existed a normogonadotrophic oligomenorrhoea with mostly low baseline urinary oestrogens in combination with moderate galactorrhoea. Other values were within normal limits.

The clinical and laboratory findings during bromergocryptine treatment are summarized

in Fig. 5. Her galactorrhoea virtually disappeared within 4 weeks, thereafter only a few drops of milk could be expressed by heavy palpation of her left breast. Exactly 4 weeks after initiation of treatment, menstrual bleeding occurred showing endometrium in the late secretory stage with pseudodecidual reaction. Thereafter her menstrual cycle was quite regular with a period of 32 days/5 days. The moderate increased pre-treatment prolactin levels were immediately suppressed to normal values within 1 week of therapy and remained so during treatment. The pre-treatment FHS and LH levels were in the normal cycle range as was the excretion of urinary oestrogens and pregnanediol. As mentioned earlier, the patient had a spontaneous menstruation at this time, her first for 10 months. During treatment the gonadotrophic hormones and also the steroids are in the normal cyclic range except for the excretion of oestrogens during the first treatment week. Lowering the dose of bromergocryptine to once daily 2·5 mg did not influence her menstrual cycle, and no increase in milk production was noticed. One month after stopping the medication her menstruation occurred on time. However, a slight increase of milk flow by palpation was noticed, mainly from her left breast.

## SIDE EFFECTS

Serious side effects were not observed in any of the three patients apart from some episodes of nausea, gastric upset and drowsiness during initiation of therapy. Within 7 days of treatment no further complaints were reported when administrating the medication during meals.

## COMMENT

In these three cases the essential role of prolactin in maintenance of non-puerperal galactorrhoea is demonstrated. The effectiveness of bromergocryptine in lowering plasma levels of prolactin is obvious. The immediate restoration of ovarian function to normal in the first two patients and probably also in the third one after return of prolactin levels to normal, strongly indicates an effect of prolactin on ovarian function. It has been postulated that the fall in prolactin levels has a stimulatory effect on the gonadotrophin-releasing factors in the hypothalamus which results in an increased secretion of gonadotrophins (Malarkey *et al.*, 1971; Besser *et al.*, 1972; Varga *et al.*, 1973; Zárate *et al.*, 1973). However, a number of cases with amenorrhoea and galactorrhoea have been reported with gonadotrophins in the normal cyclic range (Canfield & Bates, 1965; Rankin *et al.*, 1969). Zárate *et al.* (1972) tried to stimulate the ovaries in the direct *post-partum* period in lactating women by administration of gonadotrophins, but no response could be demonstrated. Based on this evidence, Zárate *et al.* (1972) and other investigators believe in an antigonadotrophic action of prolactin at the level of the ovaries (Reyes *et al.*, 1972). This fits in well with our observations in these three cases that normal plasma levels of gonadotrophic hormones are present concomitant with low normal to pathologically low levels of oestrogens. We now have evidence from a study in progress that this status also exists in normal lactating puerperal women. Recently it has been shown that prolactin has an affinity for ovarian tissue (Turkington *et al.*, 1973).

In cases of galactorrhoea and amenorrhoea due to pituitary enlargement with opthalmological or neurological complications, surgical intervention still seems the therapy of choice. If that is not necessary, progestational steroids in combination with oestrogens may

give temporary relief. Clomiphene therapy or administration of gonadotrophic hormones have shown various results in promoting fertility in these patient (Thompson & Kempers, 1965; Rankin *et al.*, 1969). In cases of gonadotrophin therapy, overstimulation of the ovaries is an additional disadvantage. Bromergocryptine indirectly restores ovarian function without overstimulation and thus promotes fertility in a physiological way. Teratological studies in rats and rabbits give no evidence of specific embryotoxic or teratogenic drug effect due to bromergocryptine (personal communication, Professor Dr E. Flückiger, Sandoz Ltd, Basle, Switzerland). Varga *et al.* (1973) reported one pregnancy while on bromergocryptine treatment with a healthy child as the outcome. Jaszmann & Sternthal (1973) reported three pregnancies during bromergocryptine medication. One had an early abortion and two others delivered healthy children after normal pregnancies (personal communication, Dr L. Jaszmann). If pregnancy is desired while on bromergocryptine medication, careful supervision is necessary with immediate cessation of medication as soon as a pregnancy is suspected, as shown in our first case. Otherwise mechanical contraceptive precautions should be recommended during drug therapy until more data have been collected concerning the teratogenicity of this drug.

In the American literature there is a considerable amount of information concerning therapy with l-dopa. This drug also effectively lowers the prolactin plasma levels and restoration of ovarian function has been reported during long-term treatment (Malarkey *et al.*, 1971; Turkington, 1972; Zárate *et al.*, 1973). However, this drug seems inferior to bromergocryptine because increasing amounts of the drug seem necessary to obtain a constant result (Besser & Edwards, 1972). In our opinion bromergocryptine is the drug of choice for treatment of the syndromes of galactorrhoea and amenorrhoea as well as for puerperal inhibition of lactation. It is still not yet clear whether long-term therapy with bromergocryptine will completely cure these patients without a rebound phenomenon after cessation of medication.

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# THE ROLE OF PROLACTIN IN THE RESTORATION OF OVARIAN FUNCTION DURING THE EARLY POST-PARTUM PERIOD IN THE HUMAN FEMALE

## I. A STUDY DURING PHYSIOLOGICAL LACTATION

RUNE ROLLAND, RUDOLF M. LEQUIN,*
LOUIS A. SCHELLEKENS AND FRANK H. DE JONG†

*Department of Obstetrics and Gynaecology. 'De Wever' Hospital, Heerlen,*
**Department of Obstetrics and Gynaecology, University Hospital, Nijmegen, and*
†*Department of Biochemistry (Division of Chemical Endocrinology),*
*Medical Faculty, Erasmus University, Rotterdam, The Netherlands*

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SUMMARY

Serial plasma levels of prolactin follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17β-oestradiol (E) and progesterone (P) were determined by radioimmunoassay in ten healthy women during late pregnancy and puer perium until the occurrence of the first menstruation, at which moment an endometrial biopsy was taken. Prolacin concentrations, which were high during late pregnancy and the early post-partum period, declined thereafter but remained above 30 ng/ml plasma during the period of breast feeding. FSH was nearly undetectable during late pregnancy and the first week post-partum but returned to normal levels between days 7 and 18. Thereafter relatively high FSH levels were found during the period of hyperprolactinaemia. Following clearance of human chorionic gonadotrophin (HCG) during the first 2 weeks post-partum, LH remained below the normal cyclic range in all cases for at least 28 days. Placental E and P were fully cleared within 7 days. In spite of relatively high levels of FSH, E remained low as did LH during the period of hyperprolactinaemia. Thereafter E increased followed by an increase of LH, suggesting a positive feedback of E on LH release. The first menses observed in five subjects during the study were preceded by (sub)-normal P levels for a relatively short period although the endometrial biopsies showed only late proliferative changes.

In spite of an increasing number of publications concerning the mechanism controlling the re-establishment of normal cyclic hypothalamic–pituitary–ovarian function following delivery, this mechanism is still not yet fully understood. The duration of post-partum amenorrhoea is extremely variable, but tends to be related to the duration of full breast-

Correspondence: Dr R. Rolland, The Milton S. Hershey Medical Center, Department of Endocrinology, The Pennsylvania State University, Hershey, Pennsylvania 17033, U.S.A.

feeding (El-Minavi & Sadek Foda, 1971; Kamal *et al.*, 1969; Berman *et al.*, 1972). During lactation the first vaginal bleeding is usually anovulatory and ovulation has not been reported before 39 days after delivery. In women whose lactation has been suppressed immediately after delivery, ovulation has been demonstrated as early as day 36 (Perez *et al.*, 1972).

Ovarian refractoriness to gonadotrophins has been suspected during the early post-partum period as vaginal smears indicated low oestrogen production in spite of normal urinary excretion of gonadotrophins (Keettel & Bradbury, 1961), and exogenously administered gonadotrophins did not give a rise in urinary excretion of oestrogens in the immediate post-partum period in lactating women (Zarate *et al.*, 1972). This could be due to an antigonadotrophic action of prolactin at the level of the ovaries as proposed by Reyes *et al.* (1972). However, Varga *et al.* (1973) believe in an antigonadotrophic action of prolactin via the hypothalamus.

The aim of the present investigation was to study the interrelationship between plasma levels of prolactin, follicle stimulating hormone, luteinizing hormone, 17$\beta$-oestradiol and progesterone in order to decide which component of the hypothalamic–hypophyseal–ovarian axis is responsible for the delayed ovulation post-partum. Human placental lactogen (HPL) was also estimated.

## SUBJECTS

Ten healthy volunteers, who had given proof of adequate lactation for more than 2 weeks in a previous puerperium, were studied. Their menstrual cycles prior to pregnancy were reported as normal with a duration of approximately 4 weeks. The pregnancies, up to the beginning of the study in the 36th week, were all uneventful.

Subjects were admitted to hospital when signs of labour were obvious. From the 36th week of pregnancy venous blood samples were drawn weekly, followed by daily collection during the first 7 days post-partum. Thereafter the samples were drawn twice weekly until the occurrence of the first vaginal bleeding recognized by the subject as a normal menstruation. Each subject then underwent an endometrial biopsy to determine the stage of endometrial development. If no vaginal bleeding had occurred within 90 days post-partum, an endometrium biopsy was performed and the study was closed.

The time interval between suckling and the drawing of the blood sample was noted, as were the number of breast feeds given each day. During the first 7 days post-partum nearly all blood samples were taken between 07.00 and 08.00 hours, thereafter always between 09.00 and 12.00 hours. The blood was collected in heparinized tubes, immediately chilled on ice, centrifuged at 2500 rev/min for 8 min and the plasma was stored at $-20°C$ until assayed. All samples from an individual subject were measured in the same assay run.

## HORMONE ESTIMATIONS

### *Protein hormones*

*Prolactin* was measured by a homologous radioimmunoassay system provided by Dr Henry Friesen, (Manitoba). The general outline of the procedure followed is described by Hwang *et al.* (1971). Minor modifications have been made by ourselves.

*FSH.* For measurement of FSH, the antiserum provided by the U.S. National Pituitary

Agency was used (Batch No. 3). For labelling with $^{125}I$, a highly purified human FSH was used (Code CPDS/2; gift from Dr W. Butt, Birmingham; biological activity 5400 IU/mg).

*LH.* The determinations of human LH were performed with an antiserum directed against intact HCG. The highly purified human LH preparation (code HLH/LN) used for iodination was prepared according to Closset *et. al* (1972). The antiserum used was carefully selected for its specificity. Highly purified FSH (CPDS/2) and less purified preparations like LER 907 and the 2nd IRP of human menopausal gonadotrophin (HMG) showed a reactivity with this antiserum which suggested that it was only the contaminating LH which was active.

*HPL.* The antiserum and highly purified HPL were obtained from Sclavo, Siena, Italy. The assay system was set up in such a way that undiluted sera could be measured directly. The antiserum is specific in that up to 140 $\mu$U human growth hormone 1st IRP and human prolactin up to 300 ng/ml do not interfere in this assay.

The $^{125}I$-labelled LH and FSH were purified prior to each assay by means of a small cellulose column (Lequin *et al.*, 1974). Separation of antibody-bound and free hormone was performed in all instances by means of the double-antibody solid phase (DASP) technique developed by Organon, Oss, the Netherlands (den Hollander *et al.*, 1972).

Calculations were done by a computer program kindly made available to us by Dr David Rodbard, National Institutes of Health, Bethesda, Md. The coefficient of variation of each sample was in all instances less than 20% and was generally between 10 and 15%.

The normal levels during a menstrual cycle of FSH and LH are shown in Table 1. In normal non-pregnant women prolactin is nearly always found below 30 ng/ml. HPL in those cases is below the limit of detection (<250 ng/ml).

TABLE 1. Normal values of FSH, LH, 17$\beta$-oestradiol and progesterone in plasma during the menstrual cycle

| Hormone | Stage of menstrual cycle | | |
|---|---|---|---|
| | Early to mid-proliferative | (Pre)ovulatory surge | Mid- to late luteal |
| FSH (mIU/ml) (2nd IRP) | 2–10 | 8–20 | 2–5 |
| LH (mIU/ml) (2nd IRP) | 10–40 | 140–500 | 10–60 |
| 17$\beta$-Oestradiol (pg/ml) | 50–100 | 200–350 | 80–150 |
| Progesterone ($\mu$g/dl) | <0·12 | | >0·50 |

*Steroid hormones*

17$\beta$-Oestradiol was measured by radioimmunoassay, using an antibody against 17$\beta$-Oestradiol-6-(0-carboxymethyl)-oxime. The antibody was a gift from Dr Exley, London, who has described its specificity in detail (Exley *et al.*, 1971). The assay procedure was described by de Jong *et al.* (1973). The Sephadex LH-20 chromatography step was omitted in these estimations. Replicate assays of distilled water, to which [$^3H$]17$\beta$-oestradiol was added, yielded a result of $6{\cdot}8 \pm 7{\cdot}8$ (s.d.) pg ($n = 72$) after correction for the mass of radioactive steroid added. The precision of the method is characterized by coefficients of variation of 10–20% for samples containing 30–150 pg 17$\beta$-oestradiol.

*Progesterone.* The concentrations of progesterone were estimated using radioimmunoassay. The method was described in detail by de Jong *et al.* (1974). Normal values for the concentration of 17β-oestradiol and progesterone found during the cycle are shown in Table 1.

## RESULTS

The duration of lactation and post-partum amenorrhoea and the stage of endometria[1] development in the subjects studied are shown in Table 2. The mean duration of lactation was 46 days with a range of 9 to more than 96 days; the mean duration of amenorrhoea was 67 days with a range of 40 to more than 96 days. The endometrial biopsies showed signs of more or less marked proliferation in eight cases, although obvious secretory activity could

TABLE 2. The duration of lactation and post-partum amenorrhoea in ten women and the stage of endometrial development at the moment of the first vaginal bleeding after delivery

| Patient | Duration of lactation (days) | Duration of post-partum amenorrhoea (days) | Endometrium development |
|---|---|---|---|
| I | 30 | 52 | Mid-proliferative |
| II | 20 | 57 | Late-proliferative |
| III | 9 | 42 | Late-proliferative |
| IV | 46 | 87 | Late-secretory |
| V | >96/96 | >96*/96 | Early-proliferative |
| VI | 35 | 65 | Late-proliferative |
| VII | >93/93 | >93*/93 | Early-proliferative |
| VIII | 26 | 60 | Late-proliferative |
| IX | >42/42 | 40 | Late-proliferative |
| X | 60 | >94*/94 | Mid-secretory |
| Mean | 46 | 67 | |
| Range | 9–>96 | 40–>96 | |

* Still amenorrhoeic.

only be demonstrated in two cases. The time interval between duration of lactation and duration of amenorrhoea was more than 34 days in both cases (patients IV and X).

Table 3 summarizes the most noteworthy changes in the hormonal status of these ten women.

Two subjects (V and VII) were amenorrhoeic for more than 90 days; during this period they still breast-fed their children. Their hormonal status during this period was quite similar and the data of subject V are shown in Fig. 1.

Subjects IV and X showed clear signs of secretory activity in their endometrial biopsies. According to the rise in plasma levels of progesterone above 0·50 μg/dl, plasma ovulation must have taken place at about day 74 in subject IV and at day 78 in subject X. Their hormonal data were quite similar during the observation time and Fig. 2 shows the data of

TABLE 3. A summary of the most noteworthy changes in the hormonal status of ten women during physiological lactation

| Patient | Duration of lactation (days) | Prolactin >30 ng/ml until day: | Ended HCG excretion at day: (approx.) | FSH>2 mIU/ml at day: (approx.) | FSH≥cyc. range (days) | LH≤cyc. range (days) | Oestradiol increase (day) | Progesterone increase (day) | Day of vaginal bleeding |
|---|---|---|---|---|---|---|---|---|---|
| I | 30 | 41 | 10 | 15 | 20–44 | 12–30 | 30 | 44 | 52 |
| II | 20 | 20 | 13 | 17 | 20–38 | 13–31 | 24 | 48 | 57 |
| III | 9 | 15 | 9 | 12 | 15–29 | 12–22 | 19 | 36 | 42 |
| IV | 46 | 49 | 11 | 18 | 32–74 | 15–60 | 58 | 78 | 87 |
| V | >96 | 96 | 15 | 18 | 29–85 | 15–60 | Not clear | None | >96 |
| VI | 35 | 35 | 11 | 18 | 25–57 | 14–66 | 39 | 57 | 65 |
| VII | >93 | 93 | 8 | 7 | 15–93 | 8–89 | None | None | >93 |
| VIII | 26 | 31 | 6 | 7 | 13–60 | 7–28 | 40 | 49 | 60 |
| IX | 42 | Not clear | 11 | 14 | 14–42 | 11–28 | 21 | None | 40 |
| X | 60 | 53 | 11 | 18 | 37–81 | 11–74 | 67 | 78 | >94 |

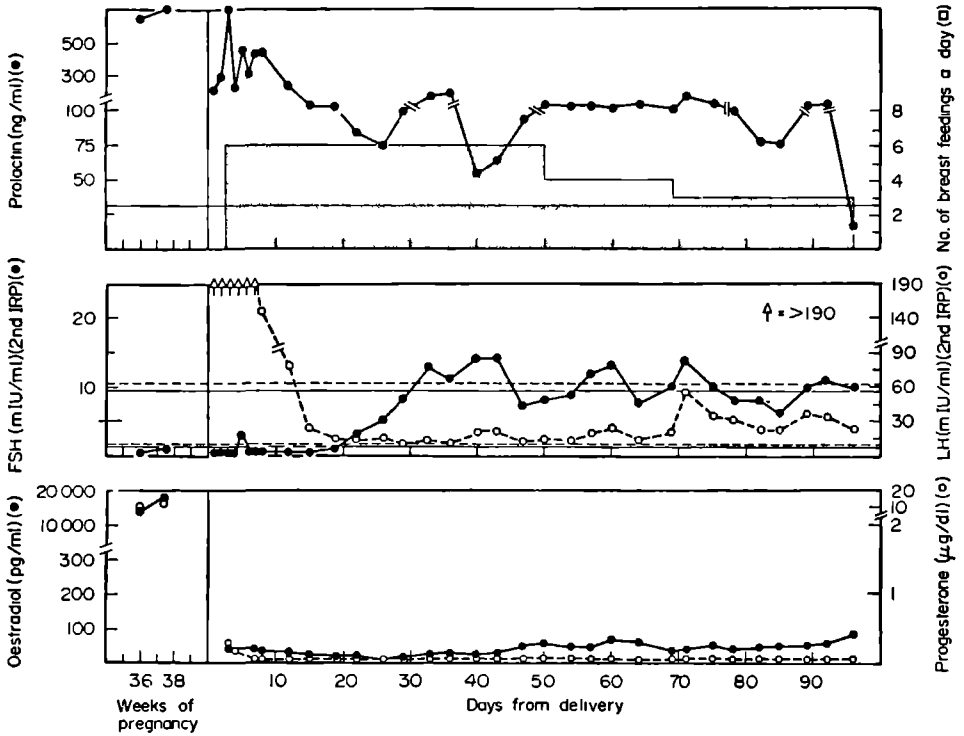


FIG. 1. Subject V. The relationship between breast-feeding, peripheral plasma levels of prolactin, FSH, LH, 17β-oestradiol and progesterone during the puerperium. (Horizontal lines indicate the range in normal cycling women.)

subject X who did not menstruate at all and, in spite of mechanical contraceptive precautions, turned out to be pregnant.

The subjects I, II, III, VI and VIII demonstrated (late) proliferative aspects in their endometrial biopsies. Their hormonal data were constantly similar with relatively high levels of FSH and low levels of LH and almost no 17β-oestradiol during the stage of hyperprolactinaemia. Before the onset of the first vaginal bleeding, always a more or less marked elevation in the peripheral levels of progesterone was noticed with a duration of less than 9 days. In Fig. 3 the hormonal data of subject II are shown to illustrate the major relationships.

Subject IX was the only one who menstruated while in full lactation. Her hormonal data are shown in Fig. 4. In spite of normal levels of FSH and LH, virtually no response in the peripheral levels of 17β-oestradiol was seen; this was in agreement with the findings of her endometrial biopsy (Table 1).

In all ten cases HPL was determined during late pregnancy and the first 3 days after delivery. Within 24 h post-partum the amount of measured HPL in all patients was below the limit of detection.

## DISCUSSION

During late pregnancy and the early post-partum period high levels of prolactin above

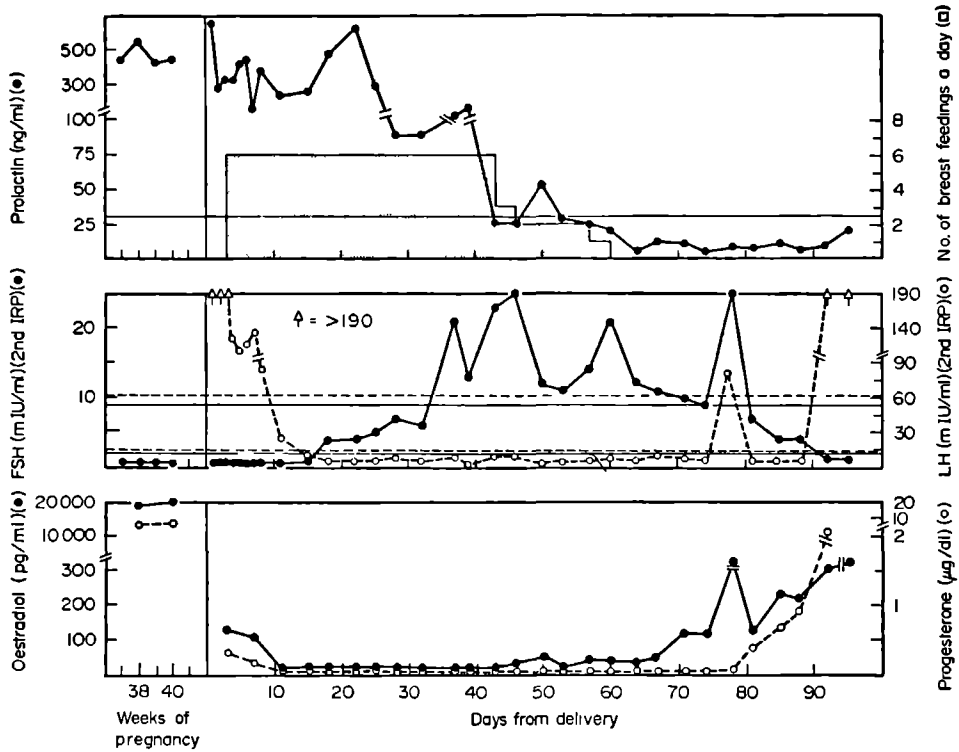


FIG. 2. Subject X. The relationship between breast-feeding, peripheral plasma levels of prolactin, FSH, LH, 17$\beta$-oestradiol and progesterone during the puerperium. (Horizontal lines indicate the range in normal cycling women.)

100 ng/ml were found; these findings are in agreement with earlier reports (Reyes *et al.*, 1972; Tyson *et al.*, 1972; Tyson & Friesen, 1973; Brun del Re *et al.*, 1973). After approximately 7 days a decrease is seen in all cases with marked fluctuations due to the varying time interval between suckling and venepuncture (Tyson *et al.*, 1972). However, as long as lactation persisted, in nine cases out of ten, prolactin levels well above 30 ng/ml were found. This is the upper level of the normal range, and the tenth patient (patient IX) demonstrated levels in the upper normal range. During the period of weaning, a steady decrease in prolactin concentrations occurred; as soon as breast-feeding had definitely been stopped, all levels were within the normal non-pregnant range below 30 ng/ml with fluctuations. The two patients with persistent lactation for more than 90 days did not show levels below approximately 60 ng/ml even though the interval between suckling and taking the blood samples ranged from 20 min to more than 4 h. This seems to disagree with an earlier report by Tyson *et al.* (1972) of normal prolactin concentrations in breast-feeding mothers 80 days post-partum. So prolactin does seem necessary both for the initiation and the prolongation of breast feeding; however, the amount necessary for the maintenance is less.

In all ten cases FSH was scarcely detectable during late pregnancy and the early post-partum period. At day 7 in two cases and between day 9 and 18 in the rest of the group, there was an increase in FSH concentration to normal cyclic levels. This is in disagreement with the findings of Crystle *et al.* (1970) and Hanson *et al.* (1970) who reported persisting

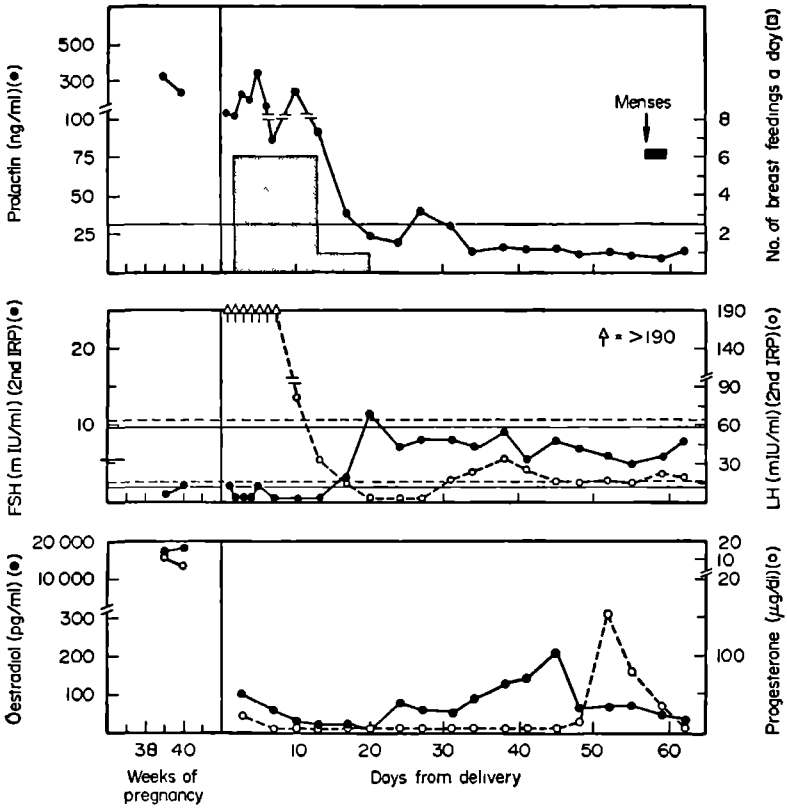


FIG. 3. Subject II. The relationship between breast-feeding, peripheral plasma levels of prolactin, FSH, LH, 17β-oestradiol and progesterone during the puerperium. (Horizontal lines indicate the range in normal cycling women.)

low levels of FSH during the first 30 days after delivery. However, the observations of Jaffe *et al.* (1969) and Reyes *et al.* (1972) support our findings.

After the increase of FSH to normal cyclic levels all patients demonstrated a further increase to levels found in the upper normal cyclic range or even above this range for approximately 30 days; thereafter there was a slow decrease to normal cyclic levels. Even in patients with persistent hyperprolactinaemia these relatively high FSH levels persisted.

The high levels of 'LH' found in the early post-partum period must be due to cross reaction in the assay system with HCG, as reported by others (Faiman *et al.*, 1968; Kell er, 1968; Jaffe *et al.*, 1969; Reyes *et al.*, 1972). This clearance of HCG and its breakd own products was completed between day 7 and day 17 (Table 3) as at that moment values of approximately 30 mIU/ml were measured. The clearance of placental 17β-oestradiol was completed much earlier and does not seem responsible for the sustained FSH depression. However, it is tempting to propose a relationship between the increase of FSH with the completed HCG clearance.

After HCG clearance, low to very low values of LH were found despite high levels of FSH in all cases. In patients with persistent lactation LH remained low for an even longer

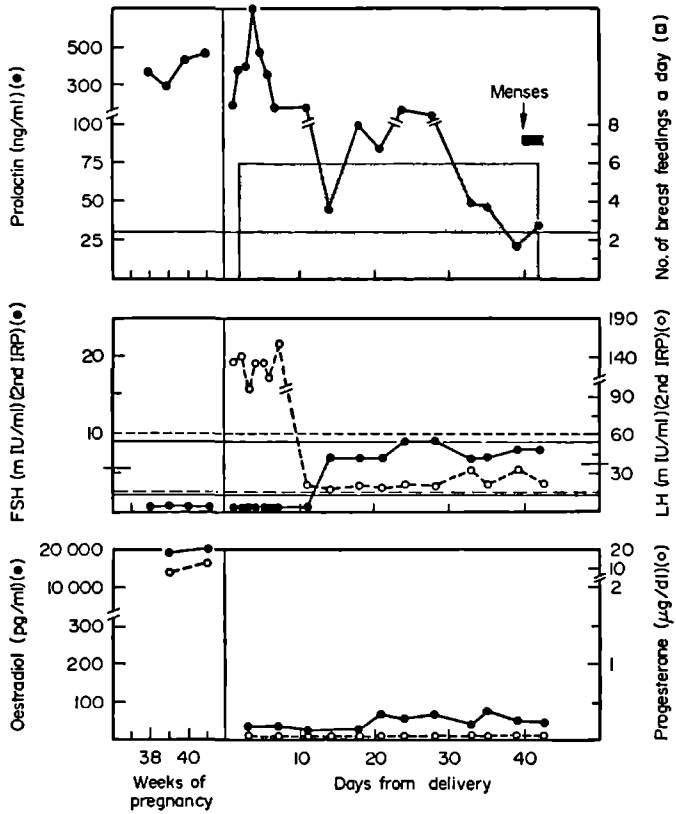


FIG. 4. Subject IX. The relationship between breast-feeding, peripheral plasma levels of prolactin, FSH, LH, 17β-oestradiol and progesterone during the puerperium. (Horizontal lines indicate the range in normal cycling women.)

period of time. The return of LH to the normal cyclic range usually occurred with a little delay, but paralleled the slight increase of 17β-oestradiol to levels found in the follicular phase. This may indicate a positive feedback of 17β-oestradiol on the hypothalamus to release luteinizing hormone-releasing hormone which is then responsible for the LH release (Ross & Vande Wiele, 1974). This is in agreement with the findings of Crystle *et al.* (1973) who observed this mechanism taking place after administration of exogenous ethinyl oestradiol at a low dose from day 10 to day 14. However, these authors thought that the same mechanism stimulated FSH release. This seems unlikely as FSH levels are restored spontaneously at exactly this time, as demonstrated in this study. Thus, in spite of relatively large amounts of FSH, the ovaries are insensitive to this stimulus so that there is no secretion of 17β-oestradiol which is necessary for the initiation of LH production. The duration of this stage seems closely related to the period of hyperprolactinaemia, and as already suggested by others (Reyes *et al.*, 1972; Zarate *et al.*, 1972; Crystle *et al.*, 1973) it strongly indicates that the ovaries are the more refractory component of the hypothalamic–pituitary–ovarian axis following delivery. This introduces an element of doubt with regard to the older hypothesis suggesting a reciprocal relationship between the secretion of gonadotro-

phins (FSH and LH) on the one hand and prolactin on the other (Sulman, 1970). In fact, prolactin has recently been shown to demonstrate affinity to ovarian tissue (Friesen, 1973, personal communication; Turkington *et al.*, 1973).

In spite of the absence of signs of secretory activity in the endometrial biopsies in five cases, more or less elevated progesterone levels were found during the last days before vaginal bleeding. This suggests that the first vaginal bleeding occurring after weaning is preceded by at least some corpus luteum function, although this may be insufficient as compared to normal corpora lutea. The endometrial biopsies in these cases do not give accurate information concerning the hormonal status at that particular moment.

## ACKNOWLEDGMENT

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# THE ROLE OF PROLACTIN IN THE RESTORATION OF OVARIAN FUNCTION DURING THE EARLY POST-PARTUM PERIOD IN THE HUMAN FEMALE

## II. A STUDY DURING INHIBITION OF LACTATION BY BROMERGOCRYPTINE

RUNE ROLLAND, FRANK H. DE JONG,*
LOUIS A. SCHELLEKENS AND RUDOLF M. LEQUIN†

*Department of Obstetrics and Gynaecology, De Wever Hospital, Heerlen,*
**Department of Biochemistry (Division of Chemical Endocrinology),*
*Medical Faculty, Erasmus University, Rotterdam, and*
†*Department of Obstetrics and Gynaecology, University Hospital, Nijmegen,*
*The Netherlands*

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SUMMARY

Serial plasma levels of prolactin follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17$\beta$-oestradiol (E) and progesterone (P) were determined by radioimmunoassay in ten healthy women during late pregnancy and the puerperium during inhibition of lactation by Bromergocryptine. This medication was continued until the occurrence of the first menstruation, at which point an endometrial biopsy was taken. Prolactin was very effectively suppressed by Bromergocryptine in all patients, as was lactation. FSH was nearly undetectable during late pregnancy and the first week post-partum with an increase back to normal levels between day 7 and day 12. Thereafter FSH levels were within the normal cyclic range. Following clearance of human chorionic gonadotrophin (HCG) during the first 2 weeks post-partum, LH was found within the normal cyclic range in all patients. From day 7 E increased in nine of ten patients to reach levels during the fourth week which are seen normally at the moment of the pre-ovulatory E-surge in cycling women. In eight of the ten cases this was concomitant with high levels of LH. The tenth patient showed a high E level at day 36. P was fully excreted within 7 days and remained low until approximately day 20. Thereafter an increase was demonstrated with levels as found during the luteal stage of the menstrual cycle in nine patients within 33 days and within 40 days in all ten patients. The endometrial biopsies showed clear signs of secretory activity. The probable action of prolactin on ovarian function is discussed. It is suggested that during the puerperium the ovaries are the more refractory part of the hypothalamic–pituitary–ovarian axis, due probably to an influence of prolactin on the ovarian steroid synthesis.

Correspondence: Dr R. Rolland, The Milton S. Hershey Medical Center, Department of Endocrinology' The Pennsylvania State University, Hershey, Pennsylvania 17033, U.S.A.

Prolactin has been thought to influence the restoration of ovarian function in the early post-partum period through an antigonadotrophic activity at the level of the ovaries (Reyes *et al.*, 1972; Zarate *et al.*, 1972). In a recent study (Rolland *et al.*, 1975) it was shown that in spite of high levels of FSH at day 10 post-partum approximately, the ovaries are insensitive to this FSH stimulus as no 17β-oestradiol is found. Plasma levels of LH were low as long as the prolactin levels were above 30 ng/ml.

Bromergocryptine (2-Br-alpha-ergocryptine; CB 154, Sandoz, Basle, Switzerland) has been shown to have a specific anti-galactic activity. Hardly any side effects of this drug have been noted. This anti-galactic activity has been shown in several experimental animal studies (Flückiger & Wagner, 1969; Billeter & Flückiger, 1971), in puerperal women (Varga *et al.*, 1972; Del Pozo *et al.*, 1972; Rolland & Schellekens, 1973), in women with galactorrhoea and menstrual disturbances (Lutterbeck *et al.*, 1971; Besser *et al.*, 1972; Jaszmann & Sternthal, 1974; Rolland *et al.*, 1974) and in men with galactorrhoea and impotence (Besser *et al.*, 1972). The drug blocks the release of prolactin from the hormone-producing pituitary cell (Pasteels *et al.*, 1971; Del Pozo *et al.*, 1973).

We decided to administer this drug during the puerperium in order to study whether inhibition of prolactin release would substantiate our hypothesis that the presence of prolactin is responsible for the delayed restoration of cyclic ovarian function in post-partum lactating women. A quicker restoration of ovarian function might therefore be expected when this drug is given.

## SUBJECTS

Ten healthy volunteers, who had given proof of adequate lactation for more than 2 weeks during a previous puerperium, were studied. Their menstrual cycles prior to pregnancy were reported as normal with a duration of approximately 4 weeks. The pregnancies were all uneventful up to the study which was started at the 36th week.

The women were admitted to hospital when signs of labour were obvious. From the 36th week of pregnancy venous blood samples were drawn weekly; then daily during the first 7 days post-partum, then twice weekly until the occurrence of the first vaginal bleeding recognized by the subject as a normal menstruation. At this stage endometrial biopsies were taken to determine the stage of endometrial development.

Within 24 h of delivery in all subjects except one (patient II) 2·5 mg Bromergocryptine was administered twice daily during breakfast and supper; this dose had proved sufficient to inhibit puerperal lactation in a previous study (Rolland & Schellekens, 1973). All subjects delivered their babies vaginally except one (patient II) who was delivered by Caesarean section; in this case, medication was started 48 h after delivery. All ten women continued to take Bromergocryptine until their first menstrual period.

During the first 7 days post-partum nearly all blood samples were taken between 07.00 and 08.00 hours, thereafter always between 09.00 and 12.00 hours. The blood was collected in heparinized tubes, immediately chilled on ice, centrifuged at 2500 rev/min for approximately 8 min and the plasma stored at −20°C until assay. In general all samples from one subject were measured in the same assay run.

## METHODS

Prolactin, HPL, FSH, LH, 17β-oestradiol and progesterone were all measured by radio-

TABLE 1. The duration of post-partum amenorrhoea and the stage of endometrial development in the puerperium in ten women during inhibition of lactation with Bromergocryptine.

| Patient | Duration of post-partum amenorrhoea (days) | Endometrium development |
|---|---|---|
| I | 32 | Late proliferative |
| II | 48 | No diagnosis |
| III | 28 | Late proliferative |
| IV | 33 | Late proliferative |
| V | 36 | Early secretory |
| VI | 33 | Mixed proliferative–secretory |
| VII | 34 | Late secretory |
| VIII | 27 | Mixed proliferative–secretory |
| IX | 43 | Pseudo decidual |
| X | 37 | Late secretory |
| Mean | 35 | |
| Range | 27–48 | |

TABLE 2. Plasma prolactin levels during late pregnancy and the early post-partum period in nine women with puerperal inhibition of lactation by Bromergocryptine

| Weeks of pregnancy | No. of cases | Plasma prolactin (ng/ml) | | |
|---|---|---|---|---|
| | | Mean | SD | Range |
| 35–36 | 6 | 312·8 | 81 4 | 203–414 |
| 37–38 | 9 | 316 4 | 61·5 | 244–447 |
| 39–40 | 8 | 338 5 | 147·1 | 178–641 |
| 41–42 | 5 | 432·6 | 173·4 | 221–670 |
| Days from delivery | | | | |
| 1 | 9 | 42·1 | 18·1 | 21–66 |
| 2 | 9 | 25·3 | 14·4 | 9–56 |
| 3 | 9 | 19·9 | 15·7 | 8–49 |
| 4 | 9 | 17 0 | 12·0 | 8–42 |
| 5 | 9 | 17·4 | 15·0 | 6–53 |
| 6 | 9 | 26 8 | 21·8 | 5–54 |
| 7 | 9 | 27·8 | 21·8 | 5–65 |
| 9–12 | 9 | 13·0 | 5·7 | 6–22 |
| 13–15 | 9 | 11·7 | 5·6 | 5–24 |
| 16–19 | 9 | 18·3 | 34·4 | 3–110 |
| 20–22 | 9 | 10·3 | 6·5 | 3–23 |
| 23–26 | 9 | 11·0 | 7·2 | 3–23 |
| 27–29 | 9 | 6·9 | 3·5 | 3–13 |
| 30–33 | 9 | 17·4 | 24·3 | 3–77 |
| 34–36 | 7 | 19 8 | 24·3 | 3–71 |
| 37–40 | 3 | 6·3 | 2·4 | 6–8 |
| 42 | 1 | 7·0 | — | — |

immunoassay. The methods used and the normal values are described in the first part of this study (Rolland *et al*, 1975).

## RESULTS

The duration of post-partum amenorrhoea and the stage of endometrial development at the moment of the first vaginal bleeding after delivery, are shown in Table 1. The mean duration of amenorrhoea was 35 days with a range of 27–48 days. Clear signs of secretory

TABLE 3 Plasma FSH levels during late pregnancy and plasma FSH and LH (HCG) levels (mIU/ml plasma 2nd IRP of human menopausal gonadotrophin (HMG)) during the early post-partum period in nine women with puerperal inhibition of lactation by Bromergocryptine

| Weeks of pregnancy | No of cases | Plasma FSH | | | Plasma LH (HCG) | | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Range | Mean | SD | Range |
| 35–36 | 6 | <1 | <1 | — | | | |
| 37–38 | 9 | <1 | <1 | — | | | |
| 39–40 | 8 | <1 | <1 | — | | | |
| 41–42 | 5 | 1·0 | 1·4 | <1–3 | | | |
| Days from delivery | | | | | | | |
| 1 | 9 | <1 | <1 | — | >200 | — | — |
| 2 | 9 | <1 | <1 | — | >200 | — | — |
| 3 | 9 | <1 | <1 | — | >200 | — | — |
| 4 | 9 | <1 | <1 | — | >200 | — | — |
| 5 | 9 | <1 | <1 | — | >200 | — | — |
| 6 | 9 | 1·7 | 2·8 | <1–7 | >200 | — | — |
| 7 | 9 | 2·8 | 3·7 | <1–8 | 193 | 156 | 42–419 |
| 9–12 | 9 | 8·0 | 3·5 | 2–12 | 76·4 | 61·3 | 21–204 |
| 13–15 | 9 | 8·2 | 3·0 | 4–14 | 41·2 | 31·3 | 18–120 |
| 16–19 | 9 | 7·7 | 1·9 | 4–10 | 36·8 | 16·4 | 19–71 |
| 20–22 | 9 | 6·2 | 2·5 | 4–11 | 34·7 | 16·7 | 14–65 |
| 23 26 | 9 | 8·0 | 3·9 | 3–15 | 65·2 | 76·2 | 11–253 |
| 27–29 | 9 | 6·9 | 5·0 | <1–19 | 36·9 | 26·9 | 10–98 |
| 30–33 | 9 | 5·4 | 1·9 | 2–8 | 37·3 | 39·1 | 9–137 |
| 34–36 | 7 | 5·3 | 3·3 | <1–9 | 23·6 | 13·6 | 8–52 |
| 37–40 | 3 | 3·8 | 4·0 | <1–8 | 21·8 | 3·5 | 18–25 |
| 42 | 1 | 3 | — | — | — | — | — |

activity were observed in six and late proliferative changes in three of the endometrial biopsies. Unfortunately no histological examination could be performed in subject II. The difference in duration of post-partum amenorrhoea in these ten women compared to ten nursing women as earlier reported (Rolland *et al.*, 1975), is statistically significant ($P<0{\cdot}001$, Student's *t*-test).

The hormonal observations in the nine cases delivered vaginally are taken together as they all show similar patterns. Their data during the observation time are shown in Tables 2–4 and Fig. 1.

*Prolactin concentrations* (Table 2, Fig. 1) were very effectively suppressed by Bromergo-

cryptine after delivery At the end of the study period an increase was sometimes noticed in the range of prolactin concurrently with a slight increase of the mean.

*FSH* (Table 3, Fig 1) was barely detectable during late pregnancy and the early post-partum period At day 6 a slight increase was noted and all women demonstrated FSH levels within the normal cyclic range at day 12 Until day 23 the mean FSH values were within the normal cyclic range; only a few levels were found above the upper normal limit. Between day 23 and 29 marked variations were observed with values clearly above the upper normal level of the proliferative or luteal stage of the cycle. Thereafter a decrease to levels as found during the normal cycle was observed. In fact, some of these FSH values found between day 23 and day 29 reached a level seen during the pre-ovulatory FSH peak in normal cyclic women.

TABLE 4 Plasma 17β-oestradiol (pg/ml plasma) and progesterone (μg/dl plasma) levels in eight women during late pregnancy and in nine women during the early post-partum period during inhibition of lactation by Bromergocryptine

| Weeks of pregnancy | No of cases | Plasma 17β-oestradiol | | | Plasma progesterone | | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Range | Mean | SD | Range |
| 35–38 | 8 | 26255 | 10283 | 9000–37800 | 17 20 | 5 40 | 9 90–25 20 |
| 39–41 | 8 | 28383 | 9948 | 12000–41200 | 19 50 | 5 90 | 14 00–31 40 |
| Days from delivery | | | | | | | |
| 3 | 9 | 153 | 75 | 63–276 | 0 40 | 0 20 | 0 16–0 75 |
| 7 | 9 | 63 | 43 | 18–140 | 0 05 | — | 0 02–0 10 |
| 9–12 | 8 | 80 | 18 | 60–112 | 0 04 | — | 0 02–0 06 |
| 13–15 | 9 | 114 | 42 | 53–180 | 0 02 | — | 0 01–0·03 |
| 16–19 | 9 | 192 | 133 | 74–495 | 0 03 | — | 0 01–0 06 |
| 20–22 | 9 | 261 | 193 | 69–661 | 0 10 | 0 22 | 0 01–0 67 |
| 23–26 | 9 | 229 | 107 | 136–438 | 0 34 | 0 54 | 0 01–1 71 |
| 27–29 | 9 | 282 | 178 | 163–633 | 0 77 | 0 71 | 0 02–1 94 |
| 30–33 | 9 | 161 | 91 | 80–358 | 1 04 | 1 00 | 0 12–2 23 |
| 34–36 | 7 | 164 | 78 | 81–288 | 0 70 | 0 50 | 0 02–1 34 |
| 37–40 | 4 | 127 | 50 | 64–180 | 0 62 | 0 32 | 0 19–0 98 |
| 42 | 1 | 178 | — | — | 0 44 | — | — |

*LH* (Table 3, Fig. 1). During the early puerperium, HCG was measured together with LH as these hormones cross react in our assay system. The interfering HCG and its metabolites were found until day 16 in some cases. Between day 16 and 23 the measured hormone concentrations were nearly within the normal cyclic range of LH with a mean value precisely within the levels found during the follicular phase of the menstrual cycle. Between day 23 and day 33 marked variations were found with an increase of the mean and also of the range of measured LH In four patients LH levels as normally seen at the moment of the pre-ovulatory LH peak were found during this period. After day 33 the mean LH value decreased and during the rest of the observation time remained at the level seen during the luteal phase of the menstrual cycle These findings correlate well with those of FSH, showing an increase of the mean and the range between day 23 and 29.

*17β-Oestradiol* (Table 4, Fig 1) which stayed high during late pregnancy, was nearly fully

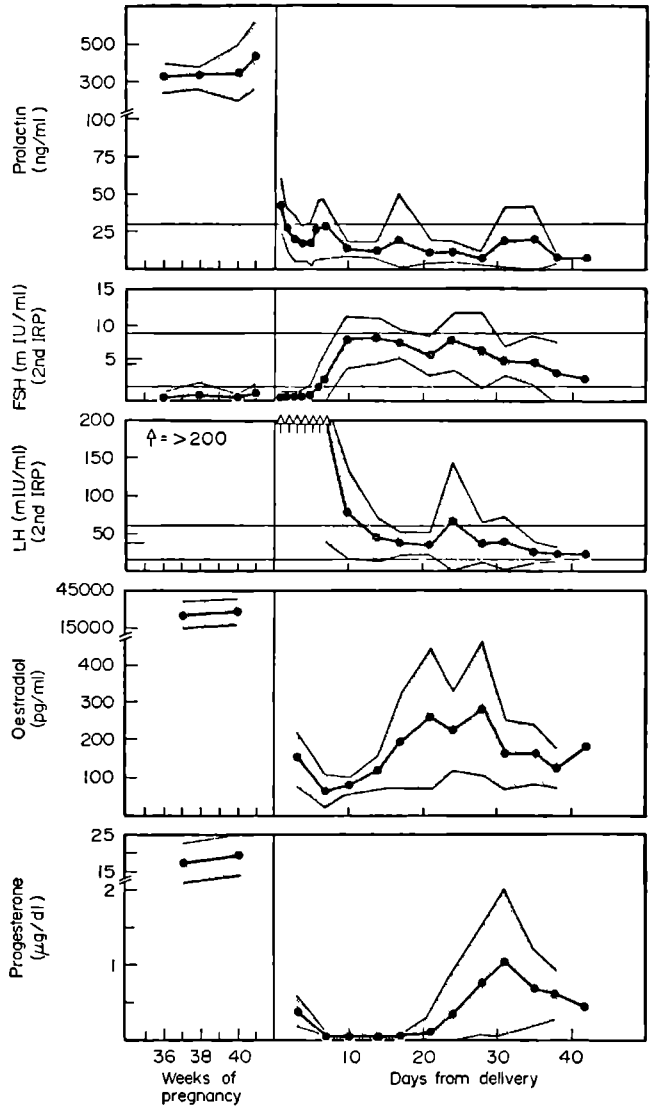


FIG. 1. The mean and SD of prolactin, FSH and LH, 17β-oestradiol and progesterone during late pregnancy and the puerperium during Bromergocryptine medication in nine healthy women. (Horizontal lines indicate normal range in cycling women.)

cleared from the circulation within 7 days of delivery. At day 7 a mean level as in the early proliferative stage of the cycle was found. Thereafter a steady increase was observed in all nine cases to concentrations equal to that found during the preovulatory 17β-oestradiol surge in normal cyclic women. In all nine cases these high levels were followed by an increase in the concentration of progesterone to above 0·12 μg/dl plasma indicating luteinization of the follicle. Subsequently the amount of 17β-oestradiol decreased in the group as a whole and remained at the level normally seen during the luteal phase of healthy cyclic women.

*Progesterone* (Table 4, Fig. 1). Within 7 days post partum, the high plasma progesterone levels observed at term decreased to nearly undetectable levels. After day 20 an increase in the mean of the progesterone levels was noted indicating that, in some cases, active corpora lutea had been formed. Eight patients showed progesterone levels above 0·50 μg/dl after day 20, the ninth showed a maximum progesterone level of 0·33 μg/dl indicating that some luteinization at least had taken place.

As the blood samples were only taken twice weekly, an ovulatory LH surge could only be found accidentally. In eight of the nine cases, however, a definite elevation of the LH level was found at a particular moment indicating that the blood sample had been taken at the start, middle or end of the ovulatory LH surge (Table 5). These 'peaks' were found on the same day as the 17β-oestradiol peak in seven cases and 2 days later in one case and were all followed by an increase in the amount of progesterone above 0·50 μg/dl plasma. The time interval between the restoration of normal FSH activity (above 2 mIU/ml) and the LH 'peak'

TABLE 5 The time course between the moment of restored FSH levels (above 2 mIU/ml plasma 2nd IRP of HMG) and the first noticed 17β-oestradiol and LH 'peak' followed by an increase in plasma progesterone levels above 0·12 μg/dl plasma in the early post-partum period in eight women with inhibition of lactation by Bromergocryptine

| Patient | A FSH within normal range at or between day(s): | B 17β-oestradiol peak at day: | C LH 'peak' at day: | Difference A−C (days) |
|---|---|---|---|---|
| III | 5 | 19 | 19 | 14 |
| IV | 7–12 | 29 | 29 | 17–22 |
| V | 9 | 27 | 27 | 18 |
| VI | 7–9 | 20 | 20 | 11–13 |
| VII | 7 | 23 | 23 | 16 |
| VIII | 7–11 | 23 | 25 | 14–18 |
| IX | 10 | 31 | 31 | 21 |
| X | 6 | 24 | 24 | 18 |

gives the approximate duration of the proliferative stage of the first menstrual cycle post-partum. In these eight cases it ranged from 11 to 22 days which is nearly within the range of normal cycling women.

The hormonal data of subject II are shown in Fig. 2. She was delivered by Caesarean section because of a contracted pelvis. Bromergocryptine was given at approximately 48 h after delivery. Unfortunately she also received during this early post-partum time drugs known to stimulate prolactin release (Promethazinum and Chlorpromethazinum). It is only at such a late stage as between day 12 and day 15 that prolactin falls below the upper normal level of 30 ng/ml plasma as found in non-pregnant women. Persistently low levels were found thereafter.

Finally the hormonal data of subject X are shown alone in Fig. 3 to serve as a model for restoration of ovarian function in the post-partum period in absence of high prolactin levels with an ovulatory LH peak at day 24 post-partum and an endometrial biopsy taken at day 37 showing clear post-ovulatory signs.

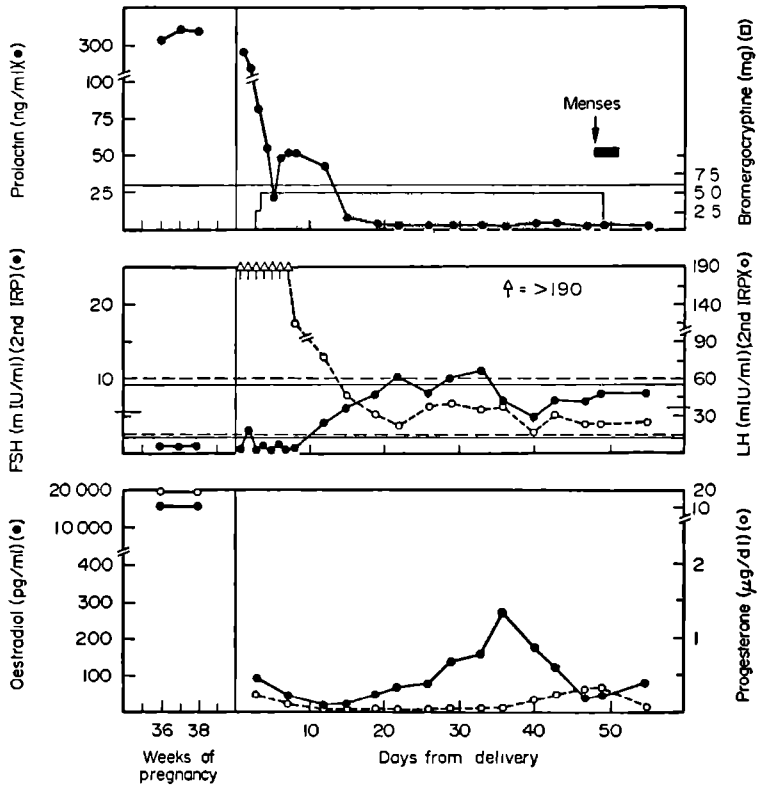


FIG. 2. Subject II. The relationship between prolactin, FSH and LH, 17β-oestradiol and progesterone during late pregnancy and the puerperium during Bromergocryptine medication. (Horizontal lines indicate normal range in cycling women.)

In all ten cases HPL was determined during late pregnancy and the first 3 days after delivery. Within 24 h post-partum the amount of measured HPL fell below the limit of detection.

## DISCUSSION

In this study the strong inhibitory action of Bromergocryptine on prolactin secretion in the early post-partum period has been demonstrated again with complete inhibition of lactation. There were no complaints of engorged or painful breasts during the study.

As in lactating women, FSH is practically indetectable during late pregnancy and the first week post-partum. Thereafter there is a return to normal cyclic levels. The timing of this increase in FSH seems to occur more discretely in the Bromergocryptine treated women, i.e. between days 7 and 10 post-partum, than in the lactating women. In the latter group the time range of the FSH surge to (supra)normal levels occurs between days 7 and 18. There was no clear correlation, however, between the time at which moment the FSH surge took place and the plasma prolactin levels at that particular moment (Rolland *et al.*, 1975). The low plasma levels of FSH during pregnancy have been thought to be due to the large

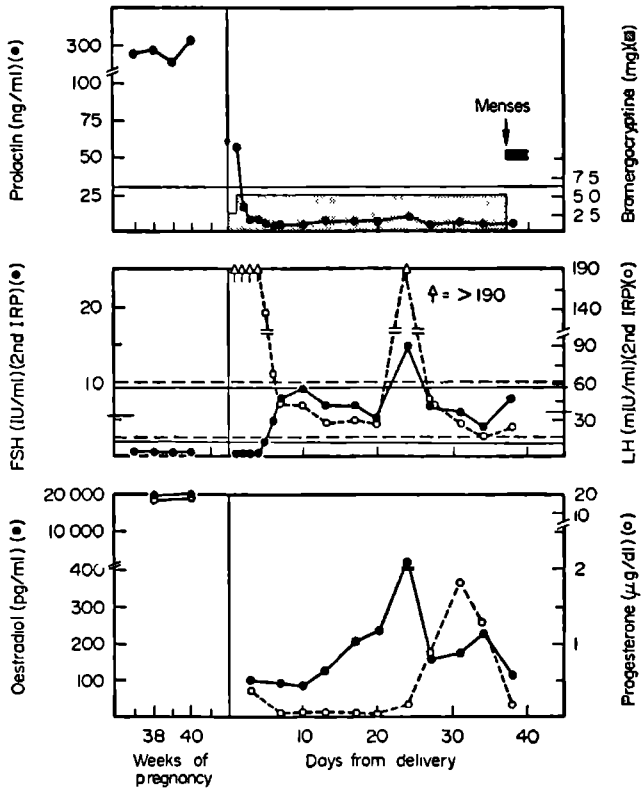


FIG. 3. Subject X. The relationship between prolactin, FSH and LH, 17β-oestradiol and progesterone during late pregnancy and the puerperium during Bromergocryptine medication. (Horizontal lines indicate normal range in cycling women.)

amount of oestrogens produced by the placenta (Jaffe *et al.*, 1969). The present study suggests, however, that FSH remains persistently low for at least 7 days post-partum in both the lactating and the Bromergocryptine treated women although 17β-oestradiol has nearly disappeared by the third day post-partum. The timing of the restoration of FSH activity, however, correlates much better with the time necessary for HCG and its metabolites to be fully cleared. It appears that in early pregnancy the opposite mechanism occurs in that FSH levels become undetectable as HCG levels increase (Jaffe *et al.*, 1969; Rolland *et al.*, 1974, 1975).

It is interesting to note that in the Bromergocryptine treated group the FSH levels return to levels as found in the normal cyclic range. This is in contrast to the post-partum FSH levels in lactating women which show clearly supra-normal FSH levels during the same period (Rolland *et al.*, 1975).

As reported by others, HCG and its metabolites are slowly cleared post-partum. This process takes approximately a fortnight (Faiman *et al.*, 1968; Jaffe *et al.*, 1969; Reyes *et al.*, 1972; Rolland *et al.*, 1975). The administration of Bromergocryptine appears not to influence this process. Thus in both groups LH may be detected only 12 days post-partum. A clear

discrepancy, however, is observed with respect to the LH levels in the lactating and Bromergocryptine treated women. In the former group LH remains scarcely detectable during the stage of hyperprolactinaemia (above 30 ng/ml), while in the latter group LH (as well as FSH) are within the range of normal cyclic women from about day 15 (day 10) post-partum onwards.

It is of particular interest that in the Bromergocryptine treated group at about day 12 post-partum, the plasma levels of 17$\beta$-oestradiol are within the limits found in the normal early pre-ovulatory phase of the menstrual cycle (Fig. 1).

This is in sharp contrast to the plasma 17$\beta$-oestradiol levels in lactating women which remain barely detectable for a long time. Thus it seems that the presence or absence or prolactin plays a major role in the restoration of normal cyclic LH levels and/or normal cyclic 17$\beta$-oestradiol levels. It is at present particularly difficult to decide at what level prolactin is exerting its inhibitory role, i.e. at the level of the ovaries or at the level of the pituitary or at both levels. The results of subject II (Fig. 2) suggest that the inhibitory effect of prolactin is at the level of the ovaries. This woman had prolactin levels above 30 ng/ml in 12–15 days, although under medication. Her LH levels decreased to normal cyclic values and remained in this range. During this period the plasma 17$\beta$-oestradiol levels remained low, at less than 50 pg/ml. It seems that only after prolactin has fallen to below approximately 30 ng/ml do the ovaries become capable of producing 17$\beta$-oestradiol in the presence of normal FSH levels. The mean values of those parameters measured in the nine other women in the Bromergocryptine treated group (Fig. 1) and the values of lactating women as earlier reported (Rolland *et al.*, 1975), support this view.

In the group of women under medication, ovulatory levels of FSH and LH were found 4 weeks post-partum (Table 3 and Fig. 1). Concomitant with these events, the plasma progesterone levels rose to levels found in the normal luteal phase of the menstrual cycle. This indicates that active corpora lutea had been formed. In the group of lactating women only one out of the ten patients studied during the same period of 40 days had progesterone levels above 0·12 $\mu$g/dl plasma indicative of luteinization of the follicle (Rolland *et al.*, 1975).

From the present study and the previous one (Rolland *et al.*, 1975) the following conclusions seem justifiable: (a) During the period of breast-feeding prolactin remains necessary. During sustained lactation, however, the amount of prolactin is much less than during the period of initiation. (b) During late pregnancy and the early post-partum period the FSH levels are nearly undetectable. They become detectable and remain in the (supra)normal range 7–14 days after delivery. This seems related more to the duration of clearance of HCG and its metabolites which take place during the first 2 weeks post-partum than to the duration of clearance of placental oestrogens which is almost complete within 72 h after delivery. (c) High levels of prolactin during the period of lactation are clearly correlated to relatively high levels of FSH and low levels of LH and 17$\beta$-oestradiol. When prolactin release from the pituitary gland is inhibited by Bromergocryptine, normal ovulatory function is immediately restored as soon as the amount of FSH reached normal cyclic levels above 2 mIU/ml plasma (2nd IRP). The increase in 17$\beta$-oestradiol seems to precede and to initiate the release of LH. This is in agreement with the findings of Crystle *et al.* (1973) who observed this mechanism taking place after administering ethinyloestradiol at a low dose from days 10 to 14 post-partum. Thus, it is tempting to assume an inhibiting influence of prolactin on ovarian steroid synthesis in humans post-partum.

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## SUMMARY AND CONCLUSIONS

### Article I:

In this paper the effect of Bromergocryptine on puerperal lactation was studied.
It was shown that inhibition of lactation could be achieved succesfully using this drug and also that already established lactation could be suppressed. However, the duration of treatment had to be approximately 21 days to prevent initiation or recurrence of lactation after stopping the drug administration. As this drug acts by blocking the release of prolactin from the pituitary gland, the essential role of prolactin on puerperal lactation has been demonstrated indirectly.

**Conclusion:**

Bromergocryptine is an effective drug for inhibition of puerperal lactation and also for suppression of already established lactation.

### Article II:

In the second paper three patients with galactorrhea due to hyperprolactinaemia and menstrual disturbances were studied.
Inhibition of prolactin release from the pituitary gland by Bromergocryptine, resulted in the disappearance of the galactorrhea with concomitant restoration of ovarian function. Laboratory findings before treatment showed elevated plasma prolactin levels, FSH and LH levels within the normal range and low to very low levels of total oestrogens in 24 hrs. urine. During treatment, prolactin concentrations decreased to values within the normal range while total urinary oestrogen excretion increased. Thereafter menstrual periods appeared in all three patients. Hormonal studies, as well as histological examination of endometrium biopsies, were indicative that ovulations did take place, which was proved by a pregnancy in one of them.

**Conclusions:**

**A:** In patients with hyperprolactinaemia, ovarian function is influenced directly or indirectly by prolactin.
**B:** Bromergocryptine is also an effective drug for the treatment of non-puerperal galactorrhea. While it gives a suppression of the milkproduction, it also restores the ovarian functions.

### Article no. III, part 1:

This part of the third paper examined the hormonal changes during the puerperium in normal lactating women.
During the period of lactation elevated peripheral plasma levels of prolactin were found in all cases. The stage of hyperprolactinaemia was characterized by relatively high plasma levels of FSH, low levels of LH and very low concentrations of oestradiol-17$\beta$ . This may be indicative of poor follicular development.
After weaning, normal levels of prolactin were found with concomitant increase in the concentrations of oestradiol-17$\beta$ and also of LH. However, there seemed to be a short

delay between the increase of oestradiol-17 $\beta$ and that of LH.
Thereafter, normal cyclic levels were found of all hormones measured. The first vaginal bleeding after delivery was mostly preceeded by a short luteal stage.

**Conclusions:**

**A:** Prolactin is necessary for both the initiation and the maintenance of puerperal lactation. Once the lactation is well established, however, the prolactin concentrations in blood decrease to lower values than during the period of initiation.
**B:** The delayed restoration of normal oestradiol-17 $\beta$ concentrations in plasma in lactating women is correlated to the time of hyperprolactinaemia.

**Article no. III, part 2:**

This part of the third paper examined the hormonal changes during the puerperium in non-lactating, Bromergocryptine treated women. The drug administration started immediately after delivery and was continued until after the occurence of the first vaginal bleeding.
Plasma prolactin concentrations were within the normal nonpregnant range within two days after delivery and remained so throughout the study. After restoration of normal plasma levels of FSH during the second week postpartum, normal concentrations of LH were found as soon as the clearance of HCG had taken place. Concomitant with the restoration of normal gonadotrophic activity, an increase in the amount of oestradiol-17 $\beta$ in plasma was found from the seventh day after delivery until high preovulatory levels were reached during the third to fourth week. At this time an increase in plasma progesterone was found and within 40 days postpartum all women showed plasma levels of this hormone indicating the presence of corpora lutea. Thus in absence of elevated prolactin levels, the ovaries are sensitive to normal gonadotrophic stimulation occuring during the second week postpartum resulting in restoration of normal ovarian functions.

**Conclusions:**

**A:** During late pregnancy and the first one to two weeks of the puerperium, plasma FSH is hardly detectable. This decrease is not correlated to the amount of prolactin present in plasma.
**B:** Inhibition of pituitary prolactin release after delivery allows the ovaries to react immediately with production of oestradiol-17 $\beta$ as soon as normal gonadotrophic activity is restored.
**C:** Women using Bromergocryptine for inhibition of puerperal lactation should be instruated to take contraceptive precautions during the puerperium because the first ovulation is likely to be anticipated through the medication.

## SAMENVATTING EN CONCLUSIES

### Artikel I:

In deze studie wordt het effect van Bromergocryptine op puerperale lactatie bestudeerd. Met dit medicament bleek het mogelijk de lactatie volledig te onderdrukken. Ook wanneer de lactatie reeds op gang was gekomen, werd het met succes toegepast. De behandeling moet echter gedurende 3 weken worden voortgezet om een recidief te voorkomen. Aangezien deze stof de afgifte van prolactine uit de hypophyse blokkeert, wordt tevens langs indirecte weg aangetoond dat prolactine een essentiële rol speelt in het mechanisme van de puerperale lactatie.

**Conclusie:**

Bromergocryptine is een werkzaam medicament wanneer de puerperale lactatie voorkomen moet worden en wanneer een reeds tot stand gekomen lactatie moet worden geremd.

### Artikel II:

In de tweede studie worden drie patiënten met een pathologische galactorrhoe en menstruatiestoornissen uitvoerig bestudeerd.
Via remming van de prolactine-afgifte uit de hypophyse met behulp van Bromergocryptine werd de pathologische galactorrhoe genezen, terwijl de ovariumfunctie zich tevens bleek te herstellen. Laboratoriumbepalingen vóór het begin van de behandeling toonden aan: een verhoogde spiegel van prolactine, FSH- en LH-waarden binnen de normale grenzen, en lage tot zeer lage spiegels van oestrogene stoffen in de 24-uurs urine. Tijdens de behandeling daalden de prolactine-spiegels tot de norm, terwijl de uitscheiding van de oestrogene stoffen in de urine steeg. Kort hierna ontstonden bij alle patiënten menstruele bloedingen. Op grond van hormoonbepalingen en op grond van het histologisch onderzoek van het endometrium kan men aannemen dat de cycli ovulatoir werden. Bij een van de patiënten werd het bewijs hiervan geleverd toen zij tijdens de behandeling zwanger werd.

**Conclusie:**

**A:** Bij patiënten met verhoogde spiegels van prolactine wordt de ovariumfunctie direct of indirect beïnvloed door het prolactine.
**B:** Bromergocryptine is tevens een werkzaam geneesmiddel bij de behandeling van niet-puerperale galactorrhoe. Het verhindert niet alleen de melkproductie, doch het leidt ook tot een herstel van de ovariumfuncties.

### Artikel III, deel 1:

In deze studie worden de hormonale veranderingen beschreven, welke werden waargenomen bij gezonde lacterende kraamvrouwen.
Bij alle vrouwen werd een verhoogde spiegel van plasma-prolactine gevonden. In deze fase werd tevens een betrekkelijk hoge spiegel gevonden van FSH, met lage waarden van LH en zeer lage waarden van oestradiol-17$\beta$ . Dit wijst op een slechte ontwikkeling van het folliculaire apparaat.
Na het staken van de borstvoeding bleken de prolactine-spiegels tot de norm te zijn

gedaald, gepaard gaande met een stijging van de concentraties van oestradiol-17 $\beta$ en LH. Het oestradiol-17 $\beta$ leek eerder te stijgen dan de LH-spiegels. In aansluiting hieraan werden van alle hormonen waarden gevonden, zoals deze voorkomen in normale cycli. De eerste vaginale bloeding na de bevalling werd meestal voorafgegaan door een korte luteale fase.

**Conclusie:**

**A:** Prolactine is noodzakelijk zowel voor het op gang brengen, als voor het onderhouden van de puerperale lactatie. Zodra de lactatie echter goed op gang is gekomen, dalen de prolactine-spiegels tot lagere waarden, dan die, welke gevonden worden in de beginperiode van de lactatie.
**B:** Het vertraagde herstel van de normale oestradiol-17 $\beta$ -spiegels in het plasma van de lacterende vrouwen is gekoppeld aan de duur van de verhoogde prolactine-spiegels.

**Artikel III, deel 2:**

Het tweede deel van het derde artikel beschrijft de hormonale veranderingen bij kraamvrouwen, die niet voedden, en die met Bromergocryptine werden behandeld. Deze behandeling begon kort na de bevalling en werd voortgezet tot na het verschijnen van de eerste vaginale bloeding.
De plasma-spiegels van prolactine daalden binnen twee dagen na de bevalling tot waarden zoals deze voor niet zwangere vrouwen gelden, en bleven gedurende de gehele studie op dit lage niveau. Nadat de spiegels van FSH in het plasma in de tweede week na de bevalling tot de norm waren gestegen, werden bovendien normale waarden voor LH gevonden zodra de uitscheiding van HCG had plaats gevonden. Tijdens het herstel van deze normale gonadotrope stimulans werd een toename gevonden van oestradiol-17 $\beta$ in het plasma, beginnend op de 7e dag post partum totdat in de 3e of 4e week post partum hoge preovulatoire waarden werden gemeten. Op dit moment werd tevens een toename van de hoeveelheid van plasma-progesteron gevonden, en binnen 40 dagen na de bevalling toonden alle vrouwen progesteron-spiegels welke wezen op een actief corpus luteum. Bij normale prolactine-spiegels blijken de ovaria dus reeds in de tweede week post partum gevoelig te zijn voor de normale gonadotrope prikkel, hetgeen resulteert in een herstel van de normale ovariumfuncties.

**Conclusie:**

**A:** Aan het einde van de zwangerschap en in de eerste veertien dagen van het kraambed kan bijna geen FSH worden aangetoond in het plasma. Deze daling van FSH is niet gecorreleerd aan de hoogte van de prolactine-spiegels.
**B:** Een remming van de verhoogde prolactine-productie na de geboorte stelt de ovaria in staat terstond te reageren op de normale gonadotrope stimulans, hetgeen blijkt uit een productie van oestradiol-17 $\beta$ .
**C:** Vrouwen die Bromergocryptine gebruiken ter onderdrukking van de puerperale lactatie moeten erop worden gewezen, dat zij terstond anti-conceptieve maatregelen moeten nemen, omdat de eerste ovulatie door deze behandeling wordt vervroegd.

## SAMMENDRAG OG KONKLUSJONER

### Artikkel I:

Denne publikasjon omhandler en undersøkelse av Bromergocryptinets innvirkning på den puerperale laktasjon.
Det ble demonstrert at Bromergocryptin både kunne hindre puerperal laktasjon og hemme allerede oppstått laktasjon. For å motvirke at det på nytt skulle oppsta laktasjon etter seponering av medikamentet var det nødvendig å behandle de første 21 dager av puerperiet. Bromergocryptin blokkerer frigjøring av prolaktin fra hypofysen - og dette hormons viktige funksjon når det gjelder puerperal laktasjon har en herved indirekte demonstrert.

**Konklusjon:**

Bromergocryptin er et medikament som effektivt hindrer laktasjon og hemmer allerede oppstått laktasjon.

### Artikkel II:

Denne publikasjon omtaler tre pasienter som hadde galaktorrhoe forårsaket av hyperprolaktinemi og menstruasjonsforstyrrelser.
Ved å motvirke frigjøring av prolaktin fra hypofysen med Bromergocryptin ble produksjonen av brystmelk hemmet samtidig som den ovarielle funksjon normaliserte seg. Laboratorieundersøkelser foretatt før behandlingen viste for høy konsentrasjon av prolaktin i plasma, normal plasmakonsentrasjon av FSH og LH og svært lav totalmengde av østrogener i døgnurin. Etter påbegynt behandling falt prolaktinkonsentrasjonen i plasma til normalt nivå samtidig som det kom til økning av den totale mengde østrogener i urinen. Noe senere menstruerte alle tre pasientene. Både hormonelle undersøkelser og histologiske undersøkelser av biopsier fra endometrium viste tydelige postovulatoriske tegn. Indikasjon på at ovulasjon hadde funnet sted var at en av kvinnene ble gravid.

**Konklusion:**

**A.** Den ovarielle funksjon hos pasienter med hyperprolaktinemi er direkte eller indirekte påvirket av prolaktin.
**B.** Bromergocryptin er også et effektivt medikament for behandling av galaktorrhoe. Samtidig som medikamentet hemmer laktasjonen, gjennopprettes den normale ovarielle funksjonen.

### Artikkel III, del 2:

Den første del av denne artikkelen omhandler undersøkelse av den hormonelle tilstand i puerperiet hos normale kvinner som ga brystmelk. Så lenge brystmelk ble gitt forble plasmakonsentrasjonen av prolaktin forhøyet. FSH - mengden i plasma ble samtidig funnet noe øket, LH-konsentrasjonen noe minsket mens østradiol-17$\beta$ -konsentrasjonen ble funnet svært lav. En slik hormonell tilstand indikerer liten eller ingen modning av de ovarielle follikler. Etter at brystmelkernæringen var stoppet ble det påvist normale verdier av prolaktin i plasma samtidig med en økning av både østradiol-17 $\beta$ og LH-konsentrasjonen. Stigningen av østradiol-17 $\beta$ konsentrasjonen syntes å begynne før LH økningen. Alle

hormonelle konsentrasjoner var etter dette innenfor normalt område for en ovulatorisk syklus. Den første postpartale blødning hos disse kvinnene etterfulgte oftest en kort luteal fase.

**Konklusjon:**

**A.** Prolaktin er nødvendig for å igangsette og opprettholde puerperal laktasjon. Når melkeproduksjonen har kommet godt i gang er plasmakonsentrasjonen av dette hormonet lavere enn like etter fødselen.
**B.** Den langsomme gjenopprettelse av normal blodkonsentrasjon av østradiol-17 $\beta$ tilsvarer den tiden en finner forøkte mengder av prolaktin i sirkulasjonen.

**Artikkel III, del 2:**

De hormonelle forandringer i puerperiet er undersøkt hos kvinner som ikke ønsket å gi brystmelk og som ble behandlet med Bromergocryptin. Medikamentet ble gitt fra like etter fødselen til den første blødning oppstod. Allerede to dager etter fødselen ble konsentrasjonen av prolaktin i plasma funnet innenfor normalverien for ikke-lakterende kvinner. Verdiene holdt seg innenfor dette området sa lenge undersøkelsen varte. Normale verdier for FSH ble funnet etter en til to uker. Normale verdier for LH så snart HCG var fullstendig utskilt. Samtidig som normale verdier ble målt av de gonadotrope hormoner, økte plasmakonsentrasjonen av østradiol-17 $\beta$ fra den syvende dag til den tredje uke i mengder som tilsvarte en preovulatorisk fase. Etter den tredje uke økte plasma-konsentrasjonen av progesteron og alle kvinnene hadde innen 40 dager etter partus plasmaverdier forenlig med aktiv corpus luteum-funksjon. Ovarene er således følsomme for stimulering av gonadotrope hormoner så snart disse har normalisert seg - dvs.en til to uker etter fødselen - dersom plasmakonsentrasjonen av prolaktin er normal.

**Konklusjon:**

**A.** Sent i graviditeten og i de to første ukene etter fødselen er FSH i plasma nesten ikke målbar. Denne reduksjonen samvarer ikke med prolaktinkonsentrasjonen i plasma.
**B.** Hemning av prolaktinfrigjøring fra hypofysen etter fødselen gjør ovarene følsomme for stimulering av de gonadotrope hormoner så snart disse har normalisert seg.
**C.** Kvinner som bruker Bromergocryptin for å hemme puerperal laktasjon bør tilrådes bruk av antikonsepsjonsmidler ved seksuelt samliv i denne tiden - da den første ovulasjonen sannsynlig vil bli framskyndet på grunn av medikamentet.

## CURRICULUM VITAE

Schrijver dezes werd in 1944 te Bergen in Noorwegen geboren. Hij behaalde in 1963 het getuigschrift "Eksamen Artium, Reallinjen" aan "Bergens Katedralskole" te Bergen in Noorwegen. In hetzelfde jaar begon hij zijn medische studies aan de Katholieke Universiteit te Nijmegen. Het kandidaatsexamen werd afgelegd in 1966, het doctoraalexamen in 1968, terwijl het artsexamen in 1970 behaald werd. In 1966 en 1967 was hij enige tijd als student-assistent verbonden aan de afdeling Pathologische Anatomie van de Medische Faculteit te Nijmegen. Na een korte periode als waarnemer in een huisartsenpraktijk, trad hij in oktober 1970 als arts-assistent in dienst van de afdeling Gynaecologie en Obstetrie van de Medische Faculteit te Nijmegen onder leiding van Prof. Dr. J.L. Mastboom om opgeleid te worden tot vrouwenarts. Vanaf februari 1972 werd deze opleiding voortgezet in het "De Wever" Ziekenhuis te Heerlen onder leiding van Dr. L.A. Schellekens, hoofd van de afdeling Gynaecologie en Obstetrie. Sedert mei 1974 is hij verbonden aan de afdelingen "Endocrinology" en "Gynecology and Obstetrics", the Milton S. Hershey Medical Center, the State University of Pennsylvania, te Hershey, Pennsylvania, U.S.A., onder leiding van Prof. C. Wayne Bardin en Prof. Vincent G. Stenger voor de periode van een jaar. Dit verblijf werd mogelijk gemaakt door een stipendium van de Stichting Z.W.O.

## STELLINGEN

1. Prolactine beinvloedt de functie van het menselijk ovarium.
(Dit proefschrift, Artikel 2 en 3)

2. Bij het joderen van prolactine met J125 voor gebruik in radioreceptor studies, verdient de lactoperoxidase methode de voorkeur boven die waarbij gebruik wordt gemaakt van Chloramine-T.
(Thorell, J.I. en Johansson, B.G. (1971) Biochim. Biophys. Acta, **251,** 363.
Franz, W.L. en Turkington, R.W. (1972) Endocrinology, **91,** 1545.
Shiu, R.P.C. en Friesen, H.G. (1974) Biochem. J., **140,** 301.)

3. De suggesties van Del Pozo **et al.** waarbij gesteld wordt, dat de oorzaak van de amenorrhoe bij patienten met galactorrhoe-amenorrhoe syndroom zonder tumor van de hypophyse boven het niveau van de hypophyse gelegen is, wordt niet ten volle gesteund door de door hen vermelde gegevens.
(Del Pozo, E., Varga, L., Wyss, H., Tolis, G., Friesen, H., Wenner, R., Vetter, L. en Vetwiller, A. (1974) J. Clin. Endocr. Metab. **39,** 18)

4. Voor kunstverlossing van een kind in achterhoofdsligging, waarbij het voorliggend deel de bekkenbodem heeft bereikt en waarbij de kleine fontanel niet naar achteren wijst, is forcipale extractie een veilige methode voor zowel moeder als kind.
(Niswander, K.R. en Gordon, M. (1973) Am. J. Obstet. Gynecol., **117,** 619.)

5. Voor bewaking van het risico-kind in utero tijdens de zwangerschap, is het Cardiotocogram van groot belang, omdat waardevolle informatie zonder tijdsverlies, moeiteloos en zonder risico voor moeder en kind verkregen kan worden.
(Beard, R.W., Filshie, G.M., Knight, C.A. en Roberts, G.M. (1971) J. Obstet. Gynaecol. Brit. Comnwlth. **78,** 865.
Tushuizen, P.B.Th., Stoot, J.E.G.M. en Ubachs, J.M.H. (1974) Am. J. Obstet. Gynecol. **119,** 638.)

6. Exconisatie vormt zelden een onmisbare schakel bij de hedendaagse diagnostiek van het carcinoma colli uteri, stadium 0.
(Schellekens, L.A. Abstracts XI International Cancer Congress, Florence, Italy, October 1974.)

7. Aan ieder ziekenhuis met een verloskundige afdeling behoort tevens verbonden te zijn een kinderarts met speciale belangstelling voor perinatologie.

8. Bij eventuele hernieuwde onderhandelingen omtrent toetreding tot de E.E.G., is de positie van Noorwegen versterkt doordat naast natuurprodukten als vis en hout, ook olie en gas aangeboden kunnen worden.

Rune Rolland

Nijmegen, 13 December 1974.